



## Full Length Article

# Effect of Combined *Lactobacillus plantarum* Inoculants on Improving Fermentation Quality and Aerobic Stability of Japanese Barnyard Millet Silage

Srivesharam Srigopalram<sup>1</sup>, Soundharrajan Ilavenil<sup>1</sup>, Hyung Soo Park<sup>1</sup>, Da Hye Kim<sup>2</sup> and Ki Choon Choi<sup>1\*</sup>

<sup>1</sup>Grassland and Forage division, National Institute of Animal Science, Rural Development Administration, Cheonan, Republic of Korea-330-801

<sup>2</sup>The United Graduate School of Agricultural Sciences, Tottori University, Japan-680-8553

\*For correspondence: choiwh@korea.kr

## Abstract

The *Lactobacillus plantarum* (LP) strains KCC-10, KCC-19, and K-46 were isolated, characterized and its probiotic skills were proved. In this experiment combination of three isolated *Lactobacillus plantarum* strains (CLP) was prepared and its fermentation potential on Japanese barnyard millet (JM) was studied. Initially, we screened the growing potential and aerobic tolerance of single LP and CLP strains in JM aqueous extract. CLP inoculants reduced the pH of JM extract well and showed high *L. plantarum* population at 48 h of anaerobic condition when compared to single LP inoculants. Further, increased lactic acid production (1215.8 mM) was noted in anaerobic CLP inoculated JM extract than single LP alone added group. Based on anaerobic fermentation results of CLP on JM aqueous extract, we further analyzed the potential of CLP on improving JM silage quality. CLP inoculated JM silage showed low (4.23) pH when compared to the non-inoculated control JM silage. Subsequently, CLP inoculated group showed a significant increase in *L. plantarum* growth ( $539.8 \times 10^6$  CFU mL<sup>-1</sup>), lactic acid (7.5 DM%) production, and reduced yeast growth than non-inoculated JM silage. The PCR results further confirmed the highest expression of *L. plantarum* gene in CLP inoculated JM silage when compared to the non inoculated group. Hence the results of this experiment confirmed the anaerobic fermentation potentials of CLP as inoculants for improving JM silage quality. © 2018 Friends Science Publishers

**Keywords:** Japanese millet; Lactic acid; *Lactobacillus plantarum*; Aerobic stability; Ensilation

## Introduction

*Echinochloa frumentacea* is the hard millet that also called as Japanese barnyard millet. The genus includes 35 species which distributed throughout the world (Verma *et al.*, 2015). Japanese barnyard millet (JM) can adapt any adverse climatic condition like high atmospheric temperature, soil with salt content and drought. Millet is the 6<sup>th</sup> cereal crop regarding world agriculture production (Devi *et al.*, 2014). The nutritive profile of millet almost equals wheat and rice. In addition millets are rich in niacin, vitamin B6, potassium, zinc, magnesium, iron, calcium, and folacin when compared to wheat and rice (Asharani *et al.*, 2010; Devi *et al.*, 2014). Fermentation of millets grains increased the nutrient bioavailability, protein quality and vitamins. Also, millet fermentation decreases the fatty acid and carbohydrates content. Fermented millet can act as probiotics delivery vehicle that offers benefits from probiotics along with whole grains for the buyer (Ilango *et al.*, 2016).

Japanese millet species are planted in countries including Japan, China, Korea, India and Central Africa (Gupta *et al.*, 2009). These millet species provide excellent

agricultural properties including animal forage and assured crop harvest. The grain of millet can be cooked like rice (Choi *et al.*, 1991; Nozawa *et al.*, 2006; Saleh *et al.*, 2013). These Japanese millet grains could be used as the drug for patients with allergic diseases (Watanabe, 1999). Hence, the importance and demand for Japanese millet as functional crop are now increasing in worldwide.

Ensilation is an important measure to avoid the weather damage when making silage (Neres *et al.*, 2013). Silages have been used as preserved cattle feed and the potential source of cattle nutrition during winter season in East Asian countries including Japan, China and Korea (Seppala *et al.*, 2016). Though silages rich in nutrients, the monosaccharide content, excess buffering ability and air removal during ensilation creates problem in quality silage making (McAllister *et al.*, 1998; Sung *et al.*, 2010a). The addition of *Lactobacillus* strains as inoculants could be helpful to solve these problems and increase the fermentation quality of silage for long time (Weinberg *et al.*, 2003). Silage preparation using probiotic *L. plantarum* strains could improve cattle health and reduce the anti-nutrient level through microbial availability.

Though JM species have excellent agricultural properties, knowledge of LP strain growth in JM aqueous extract and the role of LP additives in silo making of JM still lacks information. The objective of this study was to investigate the growth potential of *L. plantarum* inoculants (LP and CLP) in JM aqueous extract at different aerobic conditions. Based on growth results the best LP inoculant was tested for improving JM silage quality. The LP strains (KCC-10, KCC-19 and KCC-46) were isolated and characterized by our team and its potential probiotic properties were published in well-reputed journals (Arasu *et al.*, 2014; Arasu *et al.*, 2015).

## Materials and Methods

### Details of *L. plantarum* Strains used in this Study

The individual *Lactobacillus Plantarum* (LP) strains KCC-10, KCC-19, KCC-46 were isolated from *Lolium multiflorum* (Italian rye grass), *Trifolium incarnataum* (Crimson clover) and *Sesamum indicum* (sesame) respectively. The initial biochemical characterization (APIZYM and API 50CH) and 16S rRNA sequencing were made. The sequences were submitted to NCBI gene bank with accession numbers KC422325.1, KC571201.1, and KC430920.1 for KCC-10, KCC-19, and K-46 respectively. The combined form of the above three *L. Plantarum* (KCC10+KCC19+KCC46) strains was prepared according to the manufacturer protocol (Top silage private limited, Jungnong Bio Inc, South Korea). Standard strain *Lactobacillus plantarum* KACC-91067 was obtained from Korean Agricultural Culture Collection, South Korea.

### Japanese Millet Water Extract Preparation

The JM forage sample (early heading stage) was collected on September 2016 at the experimental field of National Institute of Animal Science (NIAS), RDA, Seonghwan, Korea. The collected plant material was dried and ground. Then the JM powder was sterilized using ethylene dioxide. Subsequently, 300 grams of powdered JM was mixed with 1000 mL of sterile water and kept in a shaker at 150 rpm for 24 h. Then the JM extract was consecutively sieved using well folded (15 layers) muslin cloth and the filtrate was further filtered using filter paper (Whatman No 1). Finally, the microbes in the filtrate were removed using filtration through micro pore membrane filters (0.4  $\mu$ M and 0.22  $\mu$ M) for further experiments.

### Bacterial Inoculation

The single and CLP *L. plantarum* strains were grown using MRS broth medium for 24h at 37°C. Consequently 50 mL of sterile JM extract was taken in 250 mL conical flask as triplicate for each respective experimental group. Then 10  $\mu$ L of 0.1OD bacterial cultures were loaded alone into respective conical flask containing JM extract.

Further, conical flasks with bacterial inoculums were kept in incubator (37°C and 150 rpm) for 48 h. The pH and bacterial population of the JM extract was examined every 12 h time interval. For bacterial counting, 100  $\mu$ L JM extract from each flask was taken after 48 h and ten-fold serially diluted with sterile water. Then 100  $\mu$ L of diluted sample was spread onto MRS agar and stored in incubator at 37°C for two days (Soundharrajan *et al.*, 2017).

### Estimation of Organic Acid Content

The organic acid content (lactic acid, acetic acid and butyric acid) was estimated using HPLC with UV detector (Auto sampler, Agilent 1100 Series, USA). The sample filtrate was prepared using whatman no. 6 and 0.22  $\mu$ M syringe filter. After that, the filtrate was injected to HPLC. C-18 column was used for the separation of organic acid. The total run time was 30 min and the wavelength for organic acids detection was 210 nm (Soundharrajan *et al.*, 2017).

### Japanese Millet Silage Preparation

Early heading stage of JM was collected and separated into non-inoculated and CLP inoculated group. In non inoculated group, only JM was used for fermentation analysis. However in CLP added group, 1g.kg<sup>-1</sup> of CLP was inoculated as additive for testing fermentation quality. Triplicate samples were prepared for each group and sealed tight to avoid air flow. After that the JM samples were kept at anaerobic condition for 45 days. After 45 days, the JM silage bags were opened and the silage parameters such as nutritive profile, microbial enumeration and organic acid content were estimated.

### JM Silage Nutrient Profile Analysis

The moisture content of the JM silage sample was removed by drying at 60°C in an oven for 24 h. After that the dried JM samples were powdered well for the estimation of nutritive profiles (1 mm sieve). Standard procedures were used to quantify the silage nutritive profiles such as crude protein (AOAC, 1990), Neutral detergent fiber (NDF), Acid detergent fiber (ADF) (Soset *et al.*, 1993), Total digestible nutrient (TDN) (Holland *et al.*, 1990; Sung *et al.*, 2010b) was calculated.

### JM Silage Microbial Population Enumeration

10 g of JM silage samples were soaked in 100 mL sterile distilled water and incubated in shaker at 37°C, 150 rpm for 60 min. Subsequently, tenfold serial dilution was done with water. 100  $\mu$ L of the diluted silage sample was spread on MRS agar (Difco) and Bromocresol purple blue agar. Then the plates were incubated at 36  $\pm$  1°C for 48 h for *L. plantarum* colony counting. 3 M Petri film (3 M Microbiology Products, St. Paul, USA) was used to enumerate Yeasts and molds at 28  $\pm$  1°C for 48 h.

Fungal growth was identified by spreading 100  $\mu\text{L}$  of silage sample on Potato Dextrose agar (PDA) and the plates were stored at  $28 \pm 1^\circ\text{C}$  for 72 h.

### PCR based Identification of Bacterial Strains in JM Silage Sample

Ten grams of JM silage samples were taken from each group and soaked in distilled water at  $37^\circ\text{C}$  on shaker (150 rpm) for 2 h. After incubation, the silage particles were removed by filtration through muslin cloth. The filtrate with bacterial strains was centrifuged (4000 g at  $4^\circ\text{C}$  for 30 min) and the bacterial pellets were washed three times with sterile distilled water. Then the bacterial DNA was isolated using QIAquick® kit (Qiagen Ltd., Crawley, UK). The expression patterns of lactic acid bacteria including *L. lactics*, *L. plantarum*, *L. brevis* and *P. pentosaceus* were studied using specific primers. For amplification, the PCR tubes with 1  $\mu\text{L}$  crude DNA, 1  $\mu\text{L}$  specific primers 10 pM (R and F) and 18  $\mu\text{L}$  DEPC water was used (Gene Amp PCR system 9700). Standardized PCR protocol was used as follows, 5 min at  $94^\circ\text{C}$ , 35 cycles of  $95^\circ\text{C}$  for 45 s,  $58^\circ\text{C}$  for 45s,  $72^\circ\text{C}$  for 1 min,  $72^\circ\text{C}$  for 5 min and holds at  $4^\circ\text{C}$ . Amplified DNA fragments were confirmed by agarose gel electrophoresis and the expression patterns of lactic acid bacteria in JM silage sample was quantified by qPCR. For qPCR experiment, the 96 well plates were loaded with crude DNA (1  $\mu\text{L}$ ), specific primers (1  $\mu\text{L}$ ), DEPC water (3  $\mu\text{L}$ ) and SYBR green (5  $\mu\text{L}$ ). We followed our lab standardized procedure and primers that described by (Soundharrajan *et al.*, 2017) for qPCR experiment.

### Statistical Analysis

Triplicate samples had been used in this experiment. The analysis of variance was performed using SPSS/PC (Statistical Package for the Science, V. 12.0. USA). The T-tests were used to determine treatment mean difference at the 5% probability level.

## Results

### Growth Profile Experiment in JM Extract

We primarily analyzed the growth potential and aerobic tolerance of three single LP and the combined CLP strains on JM extract under different aerobic conditions. Both single LP and CLP inoculants highly reduced the pH of JM extract in a time-dependent manner when compared to standard strain KACC at anaerobic condition (Fig. 1). The lowest pH 3.04 was noted in CLP inoculated group (Fig. 1C) at 48 h of an anaerobic condition. Further, CLP strains grew predominantly and steadily reduced the pH of the JM extract than single LP and KACC inoculated groups. Further, the *L. plantarum* colony count of single LP and CLP added JM extract (Fig. 2) at different aerobic

conditions registered maximum growth at anaerobic condition than other aerobic condition (Fig. 2C). Notably, the CLP inoculated JM extract showed maximum *L. plantarum* growth ( $276.33 \times 10^5$  CFU/mL) than single LP inoculated JM extract at 48 h of an anaerobic incubation. These results strengthen the usage of CLP inoculants in JM silage preparation.

### Organic Acid Content in JM Extract

Based on the excellent performance of LP and CLP strains in 48 h anaerobic JM extract, the organic acid content was estimated in 48 h anaerobic JM extract and compared with the results of standard strain KACC (Fig. 3). The lactic acid (203.5 mM) content was significantly ( $P < 0.05$ ) higher in CLP added group than KACC (123.5 mM) (Fig. 3D) inoculated groups. Similarly, the acetic acid content was almost two fold higher in CLP treated JM extract than single LP strains (Fig. 4). These results confirmed the fermentation potential of CLP strains in high percentage water environment that resembles silo environmental condition. Therefore, CLP used for JM silage preparation.

### Effect of CLP on Nutritive Profiles of JM Silage

Table 1 represents the nutritive profiles of experimental JM silage. The nutritive profiles, such as crude protein (CP), Acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrient (TDN) did not display significant differences from those of the non inoculated silage. Further, the moisture content result showed the slight increase in CLP group when compared to control. This result cleared that the CLP inoculants did not affect the digestibility and the original environment of JM silage.

### Organic Acid Content in JM Silage Samples

The lactic acid concentration plays an important role in deciding silage quality. Here, the addition of CLP significantly ( $P < 0.05$ ) increased the lactic acid production (7.5 DM%) when compared to the non inoculated silage (2.67 DM%). Also, the CLP group showed small quantities of acetic and no butyric acid production than non inoculated silage (Table 2).

### Microbial Population Enumeration in JM Silage Samples

Microbial populations play the central role in improving the fermentation quality of silage. Hence, we enumerated the colonies found in the experimental samples. The results are presented in (Table 3). The lactic acid bacterial counts were significantly ( $P < 0.05$ ) reduced in the non inoculated silage ( $310 \times 10^6$  CFU  $\text{g}^{-1}$ ) when compared to the CLP inoculated group ( $539.8 \times 10^6$  CFU  $\text{g}^{-1}$ ). Further, the addition of CLP reduced yeast growth than the control JM silage. Further, no fungi growth was noted in the CLP added group.

**Table 1:** Nutritive value of Japanese millet silage according to inoculation of CLP

Treatment	Moisture (%)	CP <sup>2)</sup> (%)	ADF <sup>3)</sup> (%)	NDF <sup>4)</sup> (%)	TDN <sup>5)</sup> (%)
Non inoculated	79.1	12.19	35.24	56.61	61.06
CLP <sup>1)</sup>	79.3	11.99	34.86	56.12	61.36

<sup>1)</sup>CLP: Combined *L. plantarum* <sup>2)</sup>CP: Crude protein., <sup>3)</sup>ADF: Acid detergent fiber <sup>4)</sup>NDF: Neutral detergent fiber, <sup>5)</sup>TDN: Total digestible nutrient

**Table 2:** Changes of pH and organic acids on Japanese millet silage according to inoculation of CLP

Treatment	pH	Lactic acid (DM <sup>2)</sup> %)	Acetic acid (DM%)	Butyric acid (DM%)	Flieg's score
Non inoculated	5.00 <sup>a</sup>	2.67 <sup>a</sup>	2.75	1.23 <sup>a</sup>	72
CLP <sup>1)</sup>	4.23 <sup>b</sup>	7.50 <sup>b</sup>	1.66	0.01 <sup>b</sup>	100

<sup>1)</sup>CLP: Combined *L. plantarum*, <sup>2)</sup>DM: Dry matter

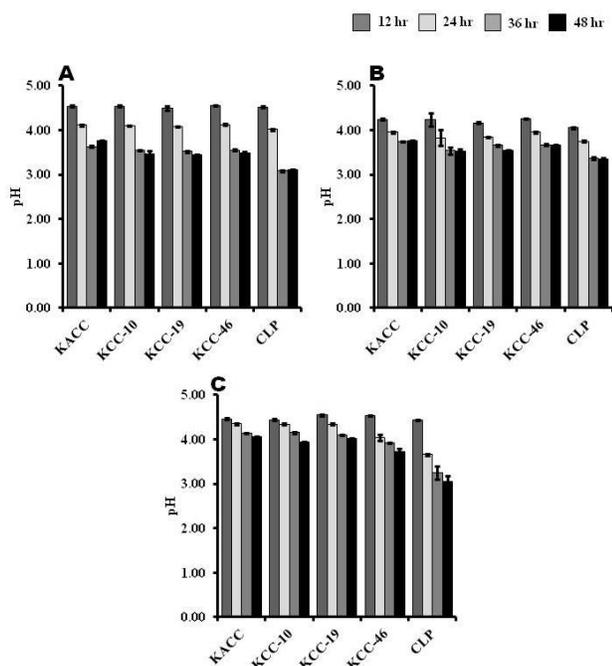
<sup>a</sup> and <sup>b</sup>: Means with different letters within a column are significantly different at the 5% level

**Table 3:** Changes of microbes on Japanese millet silage according to inoculation of CLP

Treatment	CLP (x10 <sup>6</sup> CFU <sup>2</sup> gram <sup>-1</sup> )	Yeast (x10 <sup>3</sup> CFUgram <sup>-1</sup> )	Fungi (x10 <sup>3</sup> CFUgram <sup>-1</sup> )
Non inoculated	310.0 <sup>b</sup>	45.8	0.00
CLP <sup>1)</sup>	539.8.0 <sup>a</sup>	12.8	0.00

<sup>1)</sup>CLP: Combined *L. plantarum*, <sup>2)</sup>CFU: Colony per unit

<sup>a</sup> and <sup>b</sup>: Means with different letters within a column are significantly different at the 5% level



**Fig. 1:** pH changes in KACC, KCC–10, KCC–19, K–46, and combined *L. Plantarum* (CLP) inoculated Japanese millet aqueous extract at different aerobic condition. A: Aerobic; B: Microaerobic; C: Anaerobic condition. The pH changes were noted after 12, 24, 36 and 48 h of incubation

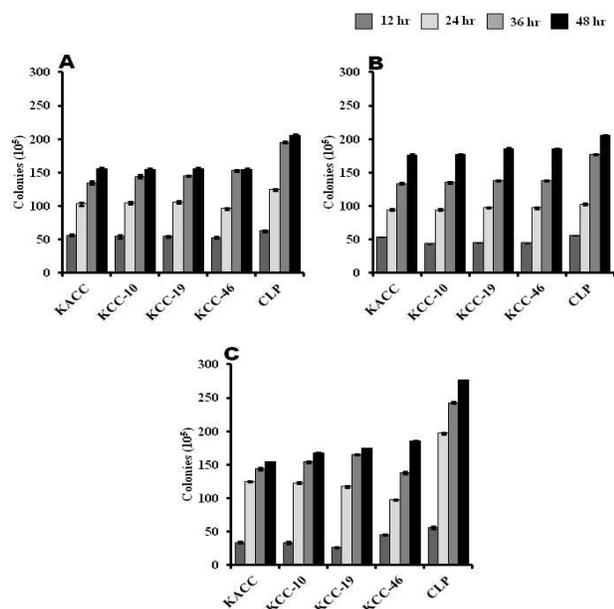
### Microbial Gene Expression Analysis by PCR Methods in JM Silage

We also analyzed lactic acid bacterial (*L. plantarum*, *L. lactis*, *L. brevis* and *P. pentosaceus*) gene expressions in the experimental silage samples using PCR technique (Fig. 5). We collected and extracted DNA from the bacterial

populations in the silage samples and identified the bacteria present in samples by using specific primers. The DNA expression patterns of the experimental samples revealed that the *L. Plantarum* gene dominantly expressed in the silage inoculated with CLP than the non inoculated silage. Other bacterial DNA, were also expressed in the silage. However, the expression patterns were similar in the control and experimental silage. This outcome confirmed the role of CLP in the successful fermentation of JM silage (Fig. 5).

### Discussion

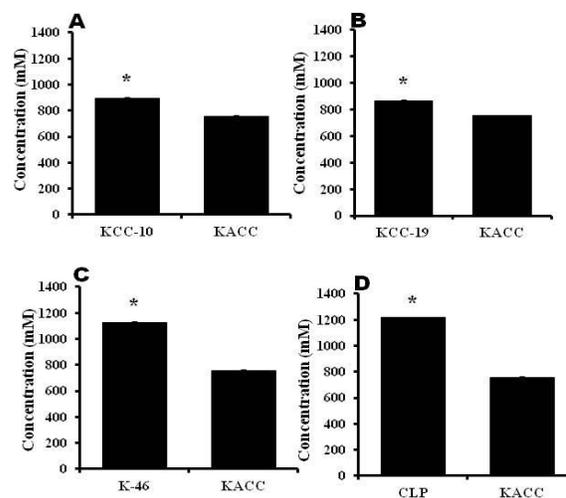
Japanese barnyard millet is the fastest growing and nutrient-rich millet. The carbohydrate content of millet is low and slowly digestible. Though JM is nutritionally richer than other cereals, yet its utilization is limited (Verma *et al.*, 2015). When compared to the concentrated animal feed, silages are cost-effective and commonly available. Hence there is a rising demand for silage preparation among farmers. However, the nutritive values of silages were changed depending on the different preparation methods (Arasu *et al.*, 2013). Accordingly, in this study, the effective inoculant was screened to improve the fermentation quality of JM forage. The addition of our single LP and CLP inoculants on JM extract reduced well the pH to the acidic level in all aerobic conditions when compared to the standard strain KACC. This result accordance with that the anaerobic condition is essential for enhancing silage quality (Ilavenil *et al.*, 2014). The highest lactic acid content of CLP added anaerobic JM extract confirmed the potential growth of Lactic acid bacteria. This result accordance with our previous report that the anaerobic tolerant lactic acid bacteria is essential for quality silage making (Srisesharam *et al.*, 2017). Hence, CLP was selected for the JM silage preparation. No significant changes were



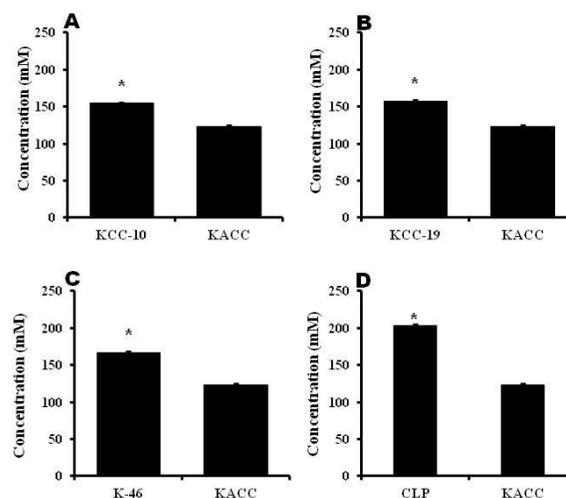
**Fig. 2:** Growth profile changes in KACC, KCC–10, KCC–19, K–46, and combined *L. Plantarum* (CLP) inoculated Japanese millet aqueous extract at different aerobic condition. A: Aerobic; B: Microaerobic; C: Anaerobic condition. The growth profiles were investigated after 12, 24, 36 and 48 h of incubation

noted in silage nutrient profile of CLP inoculated group when compared to the non-inoculated silage group. Keeping forage nutrient profile unchanged by bacterial inoculants during ensilation process confirmed the aerobic stability inoculants (Mayakrishnan *et al.*, 2014).

The crude protein level in silage is directly associated with volatile acid production and protein degradation (Danner *et al.*, 2003). Notably, in this study no significant change in CP level was noted between the experimental groups. The silage inoculation with lactic acid bacteria provided resistance to volatile acid production and preventing the silage from aerobic spoilage (Driehuis *et al.*, 2001). Many studies claimed that the addition of homofermentative lactic acid bacteria inhibits aerobic spoilage of silage and inhibit the growth of an undesirable microorganism. Indeed, organic acid production by lactic acid bacteria inoculants plays a major role in the aerobic stability of silages (Ashbell *et al.*, 2002). Similarly, CLP inoculants added experimental group showed no significant alteration in nutrient parameters like CP, ADF, NDF and TDN when compared to non inoculated control group. This confirmed possible involvement of CLP inoculants in preserving JM silage nutrient profile through extra cellular secretion like lactic acid production (Avila *et al.*, 2012; Contreras-Govea *et al.*, 2013). No butyric acid production in CLP inoculated group confirmed the low ammonia content in JM silage (Danner *et al.*, 2003). In addition, low pH level in CLP group provided unfavorable conditions for the



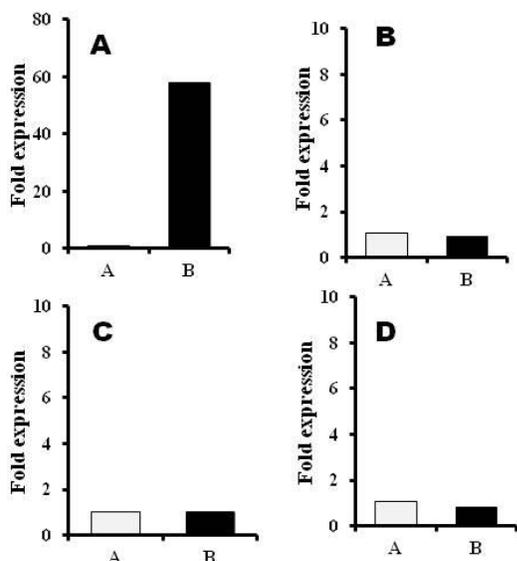
**Fig. 3:** Estimation of lactic acid content in anaerobic 48 h Japanese millet extract (JM) by HPLC. The *L. plantarum* A: KCC–10; B: KCC–19; C: K–46; D: combined *L. plantarum* (CLP) were inoculated in to JM extract and incubated under anaerobic condition for 48 h. The estimated lactic acid content was compared with standard *L. plantarum* strain KACC. \* means the statistical significance at the 5% level



**Fig. 4:** Estimation of acetic acid content in anaerobic 48 h Japanese millet extract (JM) by HPLC. The *L. plantarum* A: KCC–10; B: KCC–19; C: K–46; D: combined *L. plantarum* (CLP) were inoculated in to JM extract and incubated under anaerobic condition for 48 h. The estimated acetic acid content was compared with standard *L. plantarum* strain KACC. \* means the statistical significance at the 5% level

growth of spoilage microbes like clostridia (Muck, 2010).

The colony count of non-inoculated JM silage group showed significantly lower growth of *L. plantarum* colonies ( $310 \times 10^6$  CFUg<sup>-1</sup>) when compared



**Fig. 5:** Quantitative confirmation of microbial population through DNA expression patterns in JM silage by qPCR techniques. A–*L. plantarum*; B–*L. brevis*; C–*L. lactics*; D; *P. pentosaceus*

to CLP inoculated group ( $539.8 \times 10^6$  CFUg<sup>-1</sup>).

The increased growth of bacterial population in CLP inoculated group again confirmed the possibilities of high production of lactic acid by *L. plantarum*. High lactic acid production enhanced the silage fermentation quality with increased dry matter recovery (Filya, 2003). Also, the yeast growth significantly lowered in CLP inoculated group when compared to control silage group. Reports claimed that yeast growth in silage making process leads to aerobic deterioration of silages and thus result in the deterioration of silage production (Carvalho *et al.*, 2015). However, CLP inoculation inhibited the yeast growth with enhanced lactic acid production. Increased lactic acid production in CLP added group further confirmed the tolerance of CLP against anaerobic condition and upholding the positive environment for enhancing silage quality (Ilavenil *et al.*, 2014).

## Conclusion

The results of the present study confirmed that the addition of CLP inoculants to JM improves the fermentation profile without altering its nutrient content. Further CLP inoculants provided aerobic stability by lactic acid production and also inhibited the growth of undesirable microbes. Therefore this study suggests that CLP strains could be a useful candidate in preparing quality JM silage and further studies in CLP will be helpful in developing quality animal feed with benefits of probiotics in future.

## Acknowledgements

This work was performed with the support of the

Cooperative Research Program for Agriculture Science & Technology Development (Project title: Development of Quality Improvement and Standardization Technique for Low Moisture Storage Forage; Project No. PJ010916012017), Rural Development Administration, Republic of Korea. This study was supported by a 2014 Postdoctoral Fellowship Program of the National Institute of Animal Science, Rural Development Administration, Republic of Korea.

## References

- AOAC, 1990. Official method of analysis. *Association of Official and Analytical Chemists*, 15<sup>th</sup> edition, pp: 1–1298
- Arasu, M.V., M.W. Jung, D.H. Kim, H.S. Park, S. Ilavenil, N.A. Al-Dhabi and C.K. Choon, 2015. Identification and phylogenetic characterization of novel *Lactobacillus plantarum* species and their metabolite profiles in grass silage. *Ann. Microbiol.*, 65: 15–25
- Arasu, M.V., M.W. Jung, D.H. Kim, S. Ilavenil, M. Jane, H.S. Park, N.A. Al-Dhabi, B.T. Jeon and K.C. Choi, 2014. Enhancing Nutritional Quality of Silage by Fermentation with *Lactobacillus plantarum*. *Ind. J. Microbiol.*, 54: 396–402
- Arasu, M.V., M.W. Jung, S. Ilavenil, M. Jane, D.H. Kim, K.D. Lee, H.S. Park, T.Y. Hur, G.J. Choi and Y.C. Lim, 2013. Isolation and characterization of antifungal compound from *Lactobacillus plantarum* KCC-10 from forage silage with potential beneficial properties. *J. Appl. Microbiol.*, 115: 1172–1185
- Asharani, V.T., A. Jayadeep and N.G. Malleshi, 2010. Natural Antioxidants in Edible Flours of Selected Small Millets. *Int. J. Food Prop.*, 13: 41–50
- Ashell, G., Z.G. Weinberg, Y. Hen and I. Filya, 2002. The effects of temperature on the aerobic stability of wheat and corn silages. *J. Ind. Microbiol. Biotechnol.*, 28: 261–263
- Avila, C.L.D.S., J.C. Pinto, D.P. Oliveira and R.F. Schwan, 2012. Aerobic stability of sugar cane silages with a novel strain of *Lactobacillus* spp. isolated from sugar cane. *Rev. Bras. Zoot.*, 41: 249–255
- Carvalho, B.F., C.L.S. Avila, M.G.C.P. Miguel, J.C. Pinto, M.C. Santos and R.F. Schwan, 2015. Aerobic stability of sugar-cane silage inoculated with tropical strains of lactic acid bacteria. *Grass Forage Sci.*, 70: 308–323
- Choi, B.H., K.Y. Park and R.K. Park, 1991. Salt Tolerance and Green Fodder and Grain yields of Barnyard Millet (*Echinochloa crusgalli* var. *frumentacea*). *Kor. J. Crop Sci.*, 36: 249–253
- Contreras-Govea, F.E., R.E. Muck, G.A. Broderick and P.J. Weimer, 2013. *Lactobacillus plantarum* effects on silage fermentation and in vitro microbial yield. *Anim. Feed Sci. Technol.*, 179: 61–68
- Danner, H., M. Holzer, E. Mayrhuber and R. Braun, 2003. Acetic acid increases stability of silage under aerobic conditions. *Appl. Environ. Microbiol.*, 69: 562–567
- Devi, P.B., R. Vijayabharathi, S. Sathyabama, N.G. Malleshi and V.B. Priyadarisini, 2014. Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. *J. Food Sci. Technol.*, 51: 1021–1040
- Driehuis, F., S.J.W.H. Oude Elferink and P.G. Van Wijkelaar, 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. *Grass Forage Sci.*, 56: 330–343
- Filya, I., 2003. The effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. *J. Appl. Microbiol.*, 95: 1080–1086
- Gupta, A., V. Mahajan, M. Kumar and H.S. Gupta, 2009. Biodiversity in the barnyard millet (*Echinochloa frumentacea* Link, *Poaceae*) germplasm in India. *Genet. Resour. Crop Evol.*, 56: 883–889

- Holland, C., W. Kezar, W.P. Kautz, E.J. Lazowski, W.C. Mahanna and R. Reinhart, 1990. Pioneer forage manual: A nutritional guide. *Pioneer Hi-Bred. Int. Inc. Des Moines*, IA: 1–55
- Ilango, S., R. Pandey and U. Antony, 2016. Functional characterization and microencapsulation of probiotic bacteria from koozh. *J. Food Sci. Technol.*, 53: 977–989
- Ilavenil, S., M.V. Arasu, M. Vijayakumar, M.W. Jung, H.S. Park, Y.C. Lim and K.C. Choi, 2014. *Lactobacillus plantarum* Improves the Nutritional Quality of Italian Ryegrass with Alfalfa Mediated Silage. *J. Kor. Grassl. Forage Sci.*, 34: 174–178
- Mayakrishnan, V., I. Soundararajan, A.M. Valan, J. Min-Woong, P.H. Soo, K.J. Hea, L.Y. Cheol and C.K. Choon, 2014. Enhancement of Nutritional Quality of Italian Ryegrass Mediated Silage by Supplemented with Lactic Acid Bacteria and Chlorella. *J. Kor. Grassl. Forage Sci.*, 34: 169–173
- McAllister, T.A., R. Feniuk, Z. Mir, P. Mir, L.B. Selinger and K.J. Cheng, 1998. Inoculants for alfalfa silage: Effects on aerobic stability, digestibility and the growth performance of feedlot steers. *Livest. Sci.*, 53: 171–181
- Muck, R.E., 2010. Silage microbiology and its control through additives. *Rev. Bras. Zoot.*, 39: 183–191
- Neres, M.A., M.A. Zambom, T. Fernandes, D.D. Castagnara, J.F.H. Rodrigues, L.E. Taffarel and C.R. Javorski, 2013. Microbiological profile and aerobic stability of Tifton 85 bermudagrass silage with different additives. *Rev. Bras. Zoot.*, 42: 381–387
- Nozawa, S., M. Takahashi, H. Nakai and Y.I. Sato, 2006. Difference in SSR Variations Between Japanese Barnyard Millet (*Echinochloa esculenta*) and its Wild Relative *E. crus-galli*. *Breed. Sci.*, 56: 335–340
- Saleh, A.S.M., Q. Zhang, J. Chen and Q. Shen, 2013. Millet Grains: Nutritional Quality, Processing, and Potential Health Benefits. *Compr. Rev. Food Sci. Food Saf.*, 12: 281–295
- Seppala, A., T. Heikkila, M. Maki and M. Rinne, 2016. Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. *Grass Forage Sci.*, 71: 458–471
- Soset, P.J.V., H.G. In, D.R. Jung and R.D. Buxton, 1993. Cell wall matrix interactions and degradation session synopsis. Forage Cell Wall Structure and Digestibility. *Amer. Soc. Agron.*, 377–395
- Soundharrajan, I., D.H. Kim, S. Srisesharam, P. Kuppasamy, H.S. Park, Y.H. Yoon, W.H. Kim, Y.G. Song and K.C. Choi, 2017. Application of customised bacterial inoculants for grass haylage production and its effectiveness on nutrient composition and fermentation quality of haylage. *3. Biotech*, 7: 321
- Srisesharam, S., K. Palaniselvam, I. Soundharrajan, P. Hyung-Su, K.J. Hye, Y.Y. Hee, K.Y. Jin, J.J. Sung and C. Ki-Choon, 2017. Effect of Addition of Lactic Acid Bacteria on Fermentation Quality of *Miscanthus sinensis*. *J. Kor. Grassl. Forage Sci.*, 37: 140–144
- Sung, S., K.W. Ho, G. Jong, C. Gi-Jun, K. Ki-Yong, C. Won-Mo, P. Beom-Young and K. Young-Hoon, 2010a. Effect of Whole Crop Barley Silage Feeding on the Growth Performance, Feed Requirement and Meat Quality of Hanwoo Steers. *J. Kor. Soc. Grassl. Forage Sci.*, 30: 257–266
- Sung, S., W.H. Kim, J.G. Kim, G.J. Choi, K.Y. Kim, W.M. Cho, B.Y. Park and Y.H. Kim, 2010b. Effect of Whole Crop Barley Silage Feeding on the Growth Performance, Feed Requirement and Meat Quality of Hanwoo Steers. *J. Kor. Grassl. Forage Sci.*, 30: 257–266
- Verma, S., S. Srivastava and N. Tiwari, 2015. Comparative study on nutritional and sensory quality of barnyard and foxtail millet food products with traditional rice products. *J. Food Sci. Technol.*, 52: 5147–5155
- Watanabe, M., 1999. Antioxidative phenolic compounds from Japanese barnyard millet (*Echinochloa utilis*) grains. *J. Agric Food Chem.*, 47: 4500–4505
- Weinberg, Z.G., R.E. Muck and P.J. Weimer, 2003. The survival of silage inoculant lactic acid bacteria in rumen fluid. *J. Appl. Microbiol.*, 94: 1066–1071

(Received 03 November 2017; Accepted 28 December 2017)