Application of customised bacterial inoculants for grass haylage production and its effectiveness on nutrient composition and fermentation quality of haylage

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Abstract The present study aimed to investigate the efficacy of customised *Lactobacillus plantarum* KCC-10, KCC-19 and K-46 on nutrient composition and fermentation quality of low moisture Italian ryegrass (IRG) forage. An addition of customised bacterial inoculants (CBI) did not affect the nutrient compositions and digestibility rates of haylage. The lactic acid content was higher in CBI-inoculated haylage, whereas the amount of acetic acid and butyric acid production was significantly reduced than the control. CBI-inoculated haylage exhibited higher numbers of bacterial colonies that reduced the pH of the haylage. Low pH in haylage is an important criterion for preventing undesirable microbial growth and improves fermentation quality of haylage. PCR studies indicated that the DNA of *L. plantarum* was predominantly amplified. It evidenced that the CBI is the main reason behind the improvement of

haylage fermentation as compared to control. Overall results suggested that KCC-10, KCC-19 and K-46 are considered as potent strains for improving fermentation quality of low moisture forage and preserve its stability for a long time.

Keywords *L. plantarum* · Italian ryegrass · Low moisture forage · Nutrient and digestibility rates · Fermentation quality

Introduction

Italian ryegrass (IRG) is one of the most important forages for the ruminant animals. In South Korea, farmers always use the IRG as the main forage for the ruminant animal because of it has rich nutritional content, palatable digestibility and leaf annual forage as hay and silage during the entire season. Ensiling is an important method for making animal feed and its offers alternative ways to preserve forage grass for a long time. While ensiling, soluble carbohydrates in the samples are utilised by the microbial population that produced the higher amount of lactic acid, which reduced the pH of the forage, so it is considered as the main preservative agent in silage and haylage productions. Also, proteins were degraded into amino acids and ammonia (Whitenbury 1968; McDonald et al. 1991). The silage and haylage quality is much important due to its effects on animal production and animal health. Many biological and chemicals additives can positively affect the quality of silage. But, most of the people are using lactic acid bacteria as the additives for silage productions because they enhance lactic acid production and act as strong preservatives in ensiled materials (Varadyova et al. 2010; Ilavenil et al. 2015; Choi et al.

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2015). The fermentation is based on the competition between different groups of microbes. Lactic acid bacteria usually dominate its growth during the ensilage process. Numbers of the undesirable microorganisms are also growing and bring about anaerobic or aerobic spoilage. The *Enterococci*, *Lactococci* and *Pediococci* are initiating silage fermentation, whereas at later stages, *Lactobacillus* such as *Lactobacillus plantarum* and *Lactobacillus brevis* may dominate and replace them (Lin et al. 1992).

Early, we reported the probiotic potential and fungicidal activity of *L. plantarum* KCC-10, KCC-19 and K-46 and investigated its efficacy in silage production. The results exhibited KCC-10, KCC-19 and K-46 exhibited strong probiotic properties and antifungal activities (Valan Arasu et al. 2013, 2014a, b). KCC-10 and KCC-19 potentially improve the fermentation quality through higher production of lactic acid content (Srigopalram et al. 2015). However, excessive moisture interferes with the rapid growth of lactic acid-producing bacteria. Higher moisture in forage enhances the growth of Clostridia bacteria that is the main reason for reducing the quality and stability of forage during storage periods (Jonsson 1991; Gordon et al. 1999). Also, high moisture preparation and long-time ensiling may cause stronger acetic acid fermentation (Nishino et al. 2012). Therefore, we planned to develop the customised probiotics KCC-10, KCC-19 and K-46 and investigate their effect on low moisture forage fermentation process for ruminant animals.

Materials and methods

Isolation of probiotic bacteria

Lactobacillus plantarum KCC-10, *L. plantarum* KCC-19 and *L. plantarum* K-46 were isolated from different sources such as Italian ryegrass, Crimson clover and sesame leaf, respectively. All strains were biochemically characterised using the commercial APIZYM and API 50CH systems, biomerieux (Valan Arasu et al. 2013, 2014a, b) and the 16-s rRNA sequences were deposited at NCBI (KC422325; KC571201 and KC430920, respectively). Standard strain KACC-91067 was obtained from Korean agricultural culture collection, South Korea.

Soup preparation

Italian ryegrass soup was prepared (300 g/kg of ethylene oxide sterilised powders in 1000 mL) in sterile water and then sequentially filtered through muslin cloth, Whatman No. 1 filter paper, 0.4 and 0.22 µm membrane filters (Lab protocol).

Bacteria inoculation

KCC-10, KCC-19 and K-46 were cultured in MRS broth. All bacterial density was adjusted to 0.1 optical density (OD). From this, 10 µl of each culture individually was inoculated into 250 mL conical flask containing the different percentage of IRG soup (50 mL). Also, mixer of three strains was also inoculated. Flasks were incubated at 35 °C for 48 h. Every 12 h acidify nature and bacterial populations were checked using agar plate method. For colony counting, 1 mL from each flask was taken into 9 mL sterile water and then serially diluted with sterile water (onefold). Sample (100 µl) was spread onto MRS agar media and incubated at 35 °C for 48 h. IRG soup alone considered as control group (Arasu et al. 2014).

Customization of probiotics

KCC-10, 19 and K-46 colonies were sub-cultured individually in 250 mL conical flasks containing 50 mL MRS broth at 35 °C for 36 h. Initial optical density was adjusted to 0.1 at 600 nm. MRS broth 3000 mL was prepared and transferred into the CNS lab scale fermenter. All adjusted bacterial cultures were inoculated into a fermenter containing MRS broth (1% final concentration) and incubated at 35 °C with mild shaking (150 rpm) for 12–16 h. Well grown bacterial cultures were harvested by centrifugation and washed with suspension medium (10% Skim milk with 5% sodium glutamate). Harvested cultures were mixed with suspension medium and freeze dried (Top silage private limited, Jungnong Bio Inc, South Korea).

Haylage production

Italian ryegrass (IRG) was harvested (Flowering stage) from grassland and forage farm at National Institute of Animal Science, Cheonan, South Korea. The shoot of IRG was dried under shadow condition and chopped into small pieces (approximately 5 cm). Less than 50% moisture content of IRG was used for haylage preparation. After reaching expected moisture content, 1 kg of IRG was mixed with customised bacteria 100 g/50 ton which was dissolved in sterile distilled water and then packed in a plastic bucket without air. For control, water alone was added and incubated at room temperature for 90 days. After incubation times, these buckets were opened and analysed for nutritive values, microbial populations and fermentative metabolites in haylage (Arasu et al. 2014).

Nutritive profile analysis

Samples were powdered and filtered through a 1-mm sieve prior to analysis. The nutritive profiles such as acid

detergent fibre (ADF) and neutral detergent fibre (NDF) were analysed by Goering and Van Soest method (Goering and Van Soest 1970). Total digestible nutrient was calculated from $88.9 - (\text{ADF}\% \times 0.79)$. The crude protein (CP) content of silage was quantified (AOAC 1990).

Microbial population enumeration

Haylage (10 g) was soaked in 90 ml of sterile water in a conical flask and kept in the orbital shaker at 150 rpm for 60 min. Tenfold serial dilutions were made with sterile water. 100 µL serially diluted samples were spread on the petri disc containing selective media (Rogosa, and Sharpe agar and Bromocresol purple blue agar media). The plates were incubated at 30 ± 1 °C for 48 h. LAB colonies were counted. Yeasts and moulds were enumerated on 3 M petrifilm (3 M Microbiology Products, St. Paul, USA). Fungal growths were counted using Potato Dextrose agar (Arasu et al. 2014).

DNA extraction and quantification of DNA-based microbial population using PCRs

Ten grammes of haylage samples were taken into the sterile water from each group and kept at 37 °C for 2 h in the orbital shaker (150 rpm). Samples were filtered through the filter cloth and centrifuged at 4000 rpm for 30 min at 4 °C. Supernatant was removed and washed many times with sterile water. The genomic DNA of the experimental sample was extracted and purified using QIAquick® kit (Qiagen Ltd., Crawley, UK). For general PCR analysis (Gene Amp PCR system 9700): 1 µL crude DNA template, 1 µL of 10 pmol primers (S1 Table) and 18 µL water were added to PCR premix (maxime, Intron Biotechnology). The PCR cycles as follows initial denaturation at 94 °C, for 5 min, 29–35 cycles of denaturation at 95 °C, 45 s annealing at 57 °C, 45 s, Extension at 72 °C, 1 min, final extension at 72 °C, 5 min and holds at 4 °C. The amplified products were resolved on 1% agarose gel and the DNA band was visualised with safe view nucleic acid staining (ABM- applied biological Material). For qPCR analysis, 5 µL SYBR green mix (Applied Bio-system), 1 µL crude DNA, 1 µL of 10 pmol primers and 3 µL DEPC water (Applied Biosystem, Foster City, CA, USA). The CT values were used to analyse the fold changes in terms of relative gene expression in control and LAB cocktail treated haylage. The fold of changes were calculated using a formula $2^{-\Delta\text{CT}(\text{Target}) - \Delta\text{CT}(\text{Control})}$ (Kim et al. 2012). Cycling conditions were 95 °C for 3 min; denaturation 95 °C for 15 s; annealing/extension/plate reading at 55–60 °C for 30 s; total cycles 40; melting curve analysis 55–95 °C with 0.5 °C increment for 5 s.

Fermentative metabolites profiles

Organic acid contents were quantified by high-performance liquid chromatography with UV detector (HPLC auto sampler, Agilent 1100 Series, USA) with respective standards (Arasu et al. 2014). The samples were filtered through the filter paper (Whatman No. 6) and the filtrate was re-filtered using 0.22 µm syringe filter before injection into HPLC. Organic acids were separated on Zorbax XDB C-18 column (LC Column 250 × 4.6 mm, Agilent Technologies, USA). Following conditions were used for HPLC: mobile phase was 0.005 M sulphuric acid (pH 2.4) under isocratic elution, flow rate 0.7 ml/min, column temperature 45 °C, runtime 30 min and detected at 210 nm.

Statistical analysis

All the data were obtained from six independent replicates. Statistical difference between the control and experimental haylages was analysed by SPSS/16 software. Statistical significance was considered when *p* value was less than 0.05.

Results

Isolation and characterisation of probiotic bacteria

In the present study we used reported bacterial strains *Lactobacillus plantarum* KCC-10, *L. plantarum* KCC-19 and *L. plantarum* K-46 to investigate its efficacy on the nutritional profiles and fermentation capability in low moisture forage Italian ryegrass (IRG) during ensiling periods because most of the bacteria do not improve the fermentation process in low moisture forage.

Growth profile and its pH reducing capability

Primarily, we analysed the growth of *Lactobacillus plantarum* KCC-10, KCC-19 and K-46 individual strain as well as mixed form on different % of IRG soup at the aerobic condition. Individual and mixed bacteria were growing well and it reduces the pH of soup in a time-dependent manner (Fig. 1). *Lactobacillus plantarum* KCC-10, KCC-19 and K-46 and its combination significantly survived at 30% soup and lower the pH of the soup as compared with standard strain KACC and control. The combination of three bacterial strains was growing well and reducing the pH steadily than the single bacterial inoculation. It indicates that our strains had the potential ability to survive and minimise the pH of the IRG soup. It is the main factor for

Fig. 1 Growth profiles and pH reducing capability of *L. plantarum* KCC-10, KCC-19, K-46 and its combined form (KCC-10, KCC-19 and K-46) in different percentage of soup. The strains KCC-10, KCC-19, K-46 and its mixture were inoculated in 10, 20 and 30% IRG soup

and its growth patterns and pH in soup at 12, 24, 36 and 48 h analysed. **a** Bacterial growth and pH in 10% soup; **b** bacterial growth and pH in 20% soup; **c** bacterial growth and pH in 30% soup

using these bacterial strains for the production of the haylage.

Initial pH of the 30% soup was adjusted from 5.8 to 7.0 using 1 M NaOH and then inoculated KCC-10, KCC-19, K-46 and its mixed form. It was kept at 35 °C with mild shaking under microaerobic and anaerobic conditions. Every 12 h, we analysed bacterial growth and the pH of the soup. These results exhibited KCC-10, KCC-19 and K-46 colonies were increased in a time-dependent manner with simultaneous reduction of pH of the soup at both microaerobic and anaerobic conditions (Figs. 2, 3).

Lactic acid and acetic acid contents in soup

Lactic acid and acetic acid contents of the soup were quantified under the different conditions by HPLC. The contents of acids were higher in KCC-10, KCC-19, K-46 and its mixture inoculated haylage as compared to control, An especially lactic acid level was higher in mixture inoculated haylage as compared to single bacteria inoculated soup. The acetic acid level also increased slightly than control (Fig. 4a, b).

Preliminary results confirmed that the combination of three bacterial strains KCC-10, 19 and K-46 strongly

reduced pH of the soup than the single bacterial significantly. Therefore, we planned to make a customised product using these bacteria by lab-scale fermenter. KCC-10, 19 and K-46 colonies were subcultures individually in 250 mL conical flasks containing 50 mL MRS broth. The optical density of the bacteria was adjusted into 0.1 at 600 nm. Equal volumes of each bacterial culture were added into CNS lab-scale fermenter containing 3000 mL of MRS broth and incubated at 35 °C with mild shaking for 12–16 h. Then, well-grown bacterial cultures were lyophilized and used for haylage productions. A hundred grammes of customised bacterial inoculants (CBI) were used per 50 ton of haylage production. After the incubation period, the samples were opened and analysed for the nutritional and fermentation quality of haylage. We noted good smells from bacteria-inoculated haylage than the control. These smells might be due to the production of lactic acid and other organic acids by customised bacterial inoculants.

Effect of customised probiotics on nutritive profiles of haylage

Table 1 represents the nutrient compositions in control and customised bacterial (KCC-10, KCC-19 and K-46)

Fig. 2 growth patterns and pH reducing properties of KCC-10, KCC-19, K-46 and its combined form (KCC-10, KCC-19 and K-46) in pH (7) adjusted soup at microaerobic condition.

a Bacterial growth patterns in native soup; **b** pH reducing capability of inoculated strains in native soup **c** Bacterial growth patterns pH (7) adjusted soup; **d** pH reducing capacity of inoculated strains in pH (7) adjusted soup

inoculated haylages. The nutritive profiles such as CP, ADF, NDF, TDN and IVDMD did not show significant differences among the control and customised bacterial inoculants (CBI) supplemented haylages. The moisture content was similar in both control and experimental haylage. It indicates that the CBI did not affect the digestibility rate and its native form of haylage.

Lactic acid bacterial enumerations

The lactic acid bacterial colonies are playing a significant role in the improvement of fermentation quality of silage/haylage. Therefore, we enumerated numbers of the lactic acid bacteria found in the experimental samples and the results are given in Table 2. The lactic acid bacterial counts were recorded as 0.8 and 1200×10^6 CFU²/g/kg in control and experimental haylage, respectively. An addition of CBI to the forage reduces the yeast growth as compared to control haylage. No fungi growth was noted in control and CBI inoculated haylage.

Bacterial populations in the experimental samples were analysed using conventional PCR and qPCR techniques. Total genomic DNA was extracted from the experimental haylages as a crude form and identified the name of the bacteria present in samples using specific primers for *L. plantarum*, *L. lactis*, *L. brevis*, *E. facium*, *L. buchneri* and *P. pentosaceus*. The DNA expressions pattern of experimental samples exhibited that the *L. plantarum* DNA was amplified dominantly in the CBI-inoculated haylage as compared to control. Other bacterial DNA such as *L. lactis*, *P. pentosaceus* and *L. brevis* were also expressed additionally in the haylage. But, the DNA expressions were similar to both control and experimental haylages. Rest of bacterial DNA was not amplified. So, it confirmed that the CBI played an important role in successful fermentation of haylage as compared with control (Fig. 5).

Fermentative metabolites in haylage samples

The quality of haylage is based on the fermentative metabolites and its pH, especially lactic acid level. An

Fig. 3 growth profiles and pH reducing properties of KCC-10, KCC-19 and K-46 and its combined form (KCC-10, KCC-19 and K-46) in pH (7) adjusted soup at anaerobic condition. **a** Bacterial growth patterns in native soup; **b** pH reducing capacity of inoculated strains in native soup; **c** bacterial growth patterns pH (7) adjusted soup; **d** pH reducing capacity of inoculated strains in pH (7) adjusted soup

addition of CBI increased 229-fold lactic acid productions in haylage as compared to control. Acetic acid and the butyric acid level were significantly lesser than the control. In this study, we noted interesting factor that the acetic acid level was reduced in low moisture forage, whereas its level was increased in IRG soup conditions (Table 3).

Discussion

Ensiling is a process that preserved the nutritional quality of silage/haylage for a long time. Lactic acid bacteria supplement improved the quality of silage and it controls the undesirable microbial growth. Regarding the epiphytic microflora, microorganisms are naturally present in the forage crop, which possesses negative effect on the fermentation process, dry matter (DM) content and increased proteolysis during storage periods.

Recently, homofermentative LAB such as *L. plantarum*, *Enterococcus faecium* and *Pediococcus* spp have been used as additives for silage productions (Kung et al. 1987) and some heterofermentative bacteria such as *L. brevis*, *L.*

fermentum and *L. reuteri* (Keady and Steen 1994). Many scientists worldwide are continuously identifying the novel LAB for silage productions because of lactobacilli are very dominant microbes in the forage crops. Numbers of LAB in the natural plants are very less (Keady and Murphy 1996). It is not sufficient to dominate the other undesired microbial growth. Therefore, we need to explore the fastest growing LAB from natural sources. Previously we isolated different types of *Lactobacillus plantarum*: KCC-10, KCC-19 and K-46 from different sources and characterised. These strains showed strong antifungal and probiotics properties. Also, these strains improved the fermentation process in higher moisture forage (Valan Arasu et al. 2013, 2014a, b). In continuation of the study we investigated growth and pH lowering capability of *L. plantarum* KCC-10, KCC-19 and K-46 and its combination (KCC-10, KCC-19 and K-46) in Italian ryegrass soup. These experimental results exhibited that the KCC-10, KCC-19 and K-46 and mixture were growing well and reduced pH of the soup in a time -dependent manner at aerobic, microaerobic and anaerobic conditions. As per our expectation, the customised bacterial inoculants (CBI) improved

Fig. 4 a&b Analysis of lactic acid and acetic acid content in IRG soup by HPLC. KCC-10, KCC-19, K-46 and its mixture were inoculated in 30% soup and incubated at aerobic (a), microaerobic (b), anaerobic (c) conditions. The lactic acid and acetic acid contents were quantified by HPLC technique. * $p < 0.05$ compared to control and individual bacteria

the fermentation process in haylage in the sense of good smell. The smell might be due to higher lactic acid produced by the customised bacterial inoculants than the control. It is evidenced that the lactic acid bacteria were increased 1500-fold than the control. Also the addition of CBI reduced the number of year growth as compared with control. Yeast degrades the lactic acid when silage exposed to air during feed out or storage that increased pH of the silage and it allows the other bacteria and mould growths. These microorganisms are reducing the quality of silage (McDonald et al. 1991) In the present study, less numbers of yeast were exhibited in CBI-inoculated haylage than the control which indicated CBI suppresses the growth of yeast in haylage by its dominated growth.

An addition of CBI does not alter the cell wall components and digestibility nature significantly as compared with control. These data concluded that CBI did not affect the nutritive compositions and digestibility rate of haylage. Generally, LAB inoculants have the negligible ability to degrade the cell wall components (Kent et al. 1989). A number of the researchers have been reported that LAB inoculants did not give any positive effects on the ADF and NDF contents in the silage (Sharp et al. 1994; Weinberg and Muck 1996; Ávila et al. 2010). Besides, the supplement of LAB inoculants did not affect or improve the digestibility of silage (Ben-Dov et al. 2006). Similarly, our customised bacterial inoculants did not affect or improve the cell wall degradation and digestibility of the haylage as compared with control.

LAB inoculants during ensiling were ensured rapid and vigorous fermentation as a result of faster accumulation of lactic acid, which leads to making low pH values at earlier stages of ensiling and improved forage conservation (Cai et al. 1999). It confirmed that the LAB inoculants have the ability to ferment the water-soluble carbohydrates in the forage into acids, especially lactic acid with the small amount of acetic acid and butyric acid. Lactic acid plays an important role in the development of quality silage by inhibition of clostridia and aerobic bacterial growth by reducing the pH of silage (Cao et al. 2011). High moisture content of forage reduced the lactic acid production and it provides optimum conditions for clostridia growth that converts lactic acid and WSC (water soluble carbohydrates) into acetic, propionic, and butyric acid. Increases of propionic and butyric acid reduced the quality of silage. Decreases of butyric acid concentration in the haylage are indicating improved fermentation and reduced propionic acid and ammonia-N concentration that leads to reduced

Table 1 Effect of CBI on nutritive profiles of Italian ryegrass haylage

Treatment	Moisture (%)	CP (%)	ADF (%)	NDF (%)	TDN (%)	IVDMD (%)
Control	36.2	11.00	33.29	53.48	62.60	71.35
LAB	36.8	10.43	35.15	54.85	61.13	71.29

LAB lactic acid bacteria, CFU colony per unit, ADF acid detergent fibre, NDF neutral detergent fibre, TDN total digestible nutrients, IVDMD In-vitro dry matter digestibility

Table 2 Changes ofThe online version of this microbial population in experimental haylage

Treatment	LAB ($\times 10^6$ CFU ² /g/kg)	Yeast ($\times 10^3$ CFU/g/kg)	Fungi ($\times 10^3$ CFU/g/kg)
Control	0.8 ^b	8.3	0.00
LAB	1,200 ^a	3.7	0.0

LAB lactic acid bacteria, CFU colony per unit

^{a,b}Means with different letters within a column were significantly different at the 5% level

Fig. 5 Qualitative and quantitative analyses of microbial population in the samples through DNA expression patterns by PCR techniques. M marker, C control, T treatment. A conventional PCR, B quantitative PCR. * $p < 0.05$ compared to control haylage

protein degradation (Cao et al. 2010). In the present study, an addition of CBI to the forage increased lactic acid production (>200 fold) as compared to control, whereas

acetic acid and butyric acid concentrations were reduced in CBI-supplemented haylage as compared to control. It indicated that the CBI inoculation positively ferments the low moisture forage and inhibits undesirable microbial growth via production of the high amount of lactic acid with the limited amount of acetic acid and butyric acid. Previously, we investigated the effect of KCC-10 and KCC-19 on fermentation quality in high moisture IRG forage (>70%). The results exhibited KCC-10 and KCC-19 significantly improved fermentation quality through production of lactic acid level with less amount of acetic acid and butyric acid than the control (Srigopalram et al. 2015).

Conclusion

The degree of successful fermentation is based on the lactic acid bacteria populations and their secondary metabolite production in the forage, especially lactic acid level. In the present study, KCC-10, KCC-19, K-46 and its customised bacterial inoculants were growing well in liquid, high and low moisture forage. Also, these strains produced a significant amount of lactic acid in both forms of IRG sample. Overall results suggested that KCC-10, KCC-19 and K-46 are considered as potential strains for improvement of fermentation quality in IRG forage at all moisture conditions for long time storage.

Table 3 pH and organic acid (DM² %) content of control and CBI-inoculated haylage

Treatment	pH	Lactic acid	Acetic acid	Butyric acid
Control	6.33 ^a	0.01 ^a	0.45 ^a	0.13 ^a
LAB	4.25 ^b	2.29 ^b	0.39 ^b	0.01 ^b

LAB lactic acid bacteria, DM dry matter

^{a,b,c}Means with different letters within a column were significantly different at the 5% level

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Compliance with ethical standards

Conflict of Interest There is no conflict of interest.

References

- AOAC (1990) Official methods of analysis, 15th edn. Association of analytical chemists, Arlington
- Arasu MV, Jung M-W, Kim DH, Ilavenil S, Jane M, Park HS, Al-Dhabi NA, Jeon BT, Choi KC (2014) Enhancing nutritional quality of silage by fermentation with *Lactobacillus plantarum*. Indian J Microbiol 54(4):396–402. doi:10.1007/s12088-014-0473-9
- Ávila CLdS, Valeriano AR, Pinto JC, Figueiredo HCP, Rezende AVd, Schwan RF (2010) Chemical and microbiological characteristics of sugar cane silages treated with microbial inoculants. R Bras Zootec 39:25–32. doi:10.1590/S1516-35982010000100004
- Ben-Dov E, Shapiro OH, Siboni N, Kushmaro A (2006) Advantage of using inosine at the 3' termini of 16S rRNA gene universal primers for the Study of Microbial Diversity. Appl Environ Microbiol 72(11):6902–6906. doi:10.1128/AEM.00849-06
- Cai Y, Benno Y, Ogawa M, Kumai S (1999) Effect of applying lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. J Dairy Sci 82(3):520–526. doi:10.3168/jds.S0022-0302(99)75263-X
- Cao Y, Takahashi T, K-i Horiguchi, Yoshida N (2010) Effect of adding lactic acid bacteria and molasses on fermentation quality and in vitro ruminal digestion of total mixed ration silage prepared with whole crop rice. Grassl Sci 56(1):19–25. doi:10.1111/j.1744-697X.2009.00168.x
- Cao Y, Cai Y, Takahashi T, Yoshida N, Tohno M, Uegaki R, Nonaka K, Terada F (2011) Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and in vitro ruminal digestion of vegetable residue silage. J Dairy Sci 94(8):3902–3912. doi:10.3168/jds.2010-3623
- Choi K, Ilavenil S, Valan Arasu M, Park H, Kim W (2015) Effect of addition of chlorella and lactic acid bacteria on nutritive value and fermentation quality of fresh rice straw silage. Korean Soc Grassl Forage Sci 35:159–165. doi:10.5333/KGFS.2015.35.2.159
- Goering H, Van Soest P (1970) Forage fibre analysis. Handbook, U.S Gov. Print. Office, Washington, DC, Agric
- Gordon FJ, Dawson LER, Ferris CP, Steen RWJ, Kilpatrick DJ (1999) The influence of wilting and forage additive type on the energy utilisation of grass silage by growing cattle. Anim Feed Sci Technol 79(1–2):15–27. doi:10.1016/S0377-8401(99)00013-9
- Ilavenil S, Srigopalram S, Park H, Kim W, Lee K, Choi K (2015) Beneficial effects of lactic acid bacteria inoculation on oat based silage in South Korea. Korean Soc Grassl Forage Sci 35:207–211
- Jonsson A (1991) Growth of *Clostridium tyrobutyricum* during fermentation and aerobic deterioration of grass silage. J Sci Food Agric 54(4):557–568. doi:10.1002/jsfa.2740540407
- Keady TWJ, Murphy JJ (1996) Effects of inoculant treatment on ryegrass silage fermentation, digestibility, rumen fermentation, intake and performance of lactating dairy cattle. Grass Forage Sci 51(3):232–241. doi:10.1111/j.1365-2494.1996.tb02058.x
- Keady TWJ, Steen RWJ (1994) Effects of treating low dry-matter grass with a bacterial inoculant on the intake and performance of beef cattle and studies on its mode of action. Grass Forage Sci 49(4):438–446. doi:10.1111/j.1365-2494.1994.tb02021.x
- Kent BA, Arambel MJ, Winsryg MD, Walters JL (1989) Microbial inoculation of alfalfa haylage: ensiling characteristics and milk production response when fed to early lactation dairy cows. J Dairy Sci 72(9):2325–2330. doi:10.3168/jds.S0022-0302(89)79364-4
- Kim ET, Kim CH, Min KS, Lee SS (2012) Effects of plant extracts on microbial population, methane emission and ruminal fermentation characteristics in in vitro. Asian-Australas J Anim Sci 25(6):806–811. doi:10.5713/ajas.2011.11447
- Kung L Jr, Satter LD, Jones BA, Genin KW, Sudoma AL, Enders GL Jr, Kim HS (1987) Microbial inoculation of low moisture alfalfa silage. J Dairy Sci 70(10):2069–2077. doi:10.3168/jds.S0022-0302(87)80255-2
- Lin C, Bolsen KK, Brent BE, Fung DYC (1992) Epiphytic lactic acid bacteria succession during the pre-ensiling and ensiling periods of alfalfa and maize. J Appl Bacteriol 73(5):375–387. doi:10.1111/j.1365-2672.1992.tb04992.x
- McDonald P, Henderson AR, Heron SJ (1991) The biochemistry of silage. Chalcombe Publications, Lincoln
- Nishino N, Li Y, Wang C, Parvin S (2012) Effects of wilting and molasses addition on fermentation and bacterial community in guinea grass silage. Lett Appl Microbiol 54(3):175–181. doi:10.1111/j.1472-765X.2011.03191.x
- Sharp R, Hooper PG, Armstrong DG (1994) The digestion of grass silages produced using inoculants of lactic acid bacteria. Grass Forage Sci 49(1):42–53. doi:10.1111/j.1365-2494.1994.tb01975.x
- Srigopalram S, Ilavenil S, Vijayakumar M, Park HS, Lee KD, Choi KC (2015) Addition of Novel *Lactobacillus plantarum* KCC-10 and KCC-19 to improve fermentation quality and characterization of Italian ryegrass silage. J Korean Soc Grassl Forage Sci 35:195–200. doi:10.5333/KGFS.2015.35.3.195
- Valan Arasu M, Jung MW, Ilavenil S, Jane M, Kim DH, Lee KD, Park HS, Hur TY, Choi GJ, Lim YC, Al-Dhabi NA, Choi KC (2013) Isolation and characterization of antifungal compound from *Lactobacillus plantarum* KCC-10 from forage silage with potential beneficial properties. J Appl Microbiol 115(5):1172–1185. doi:10.1111/jam.12319
- Valan Arasu M, Kim Da Hye, Kim Pyoung Il, Jung Min Woong, Ilavenil Soundarajan, Jane Mariamichael, Lee Kyung Dong, Al-Dhabi Naif Abdullah, Choi Ki Choon (2014a) In vitro antifungal, probiotic and antioxidant properties of novel *Lactobacillus plantarum* K46 isolated from fermented sesame leaf. Ann Microbiol 64(3):1333–1346. doi:10.1007/s13213-013-0777-8
- Valan Arasu MJMW, Kim DH, Ilavenil S, Lee KD, Choi GJ, Al-Dhabi NA, Choi KC (2014b) Isolation and characterization of *Lactobacillus plantarum* KCC-19 from crimson silage. J Pure Appl Microbiol 8:3575–3587
- Varadyova Z, Kišidayova S, Laukova A, Jalč D (2010) Influence of inoculated maize silage and sunflower oil on the in vitro fermentation, ciliate population and fatty acid outputs in the rumen fluid collected from sheep. Czech J Anim Sci 55:105–115
- Weinberg ZG, Muck RE (1996) New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiol Rev 19(1):53–68. doi:10.1016/0168-6445(96)00025-3
- Whitenbury R (1968) Microbiology of grass silage. Process biochemistry. Grampian Press Ltd, London, p 6