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Inoculation of Potato Pulp with Antibacterial Lactic Acid Bacterium To Improve The Quality of Livestock Feed

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Abstract

Potato pulp was inoculated either with the antibacterial lactic acid bacteria *Lactococcus lactis* and *L. diolivorans* and the inoculated potato pulp was ensiled under anaerobic conditions for 30 d at room temperature in a mini-silo. We have previously reported that *L. diolivorans* produces antimicrobial peptides with potent antibacterial activity; therefore, the bacterium is expected to increase the fermentation quality of the potato pulp. The quality of the potato pulp silage was evaluated. The moisture content of the potato pulp silage was remained 822 g/kg before and after ensiling. The protein content in the silage increased from an initial concentration of 39 to 57 g/kg and 58 g/kg for *L. lactis* and *L. diolivorans* inoculations, respectively. The lactic acid content significantly increased from 2 to 52 g/kg (*L. lactis*) and 50 g/kg (*L. diolivorans*) after ensiling, whereas, toxic butyric acid was not detected with either treatment. These results suggest that the inoculation of potato pulp with *L. lactis* or *L. diolivorans* increases the quality and nutrition of potato pulp as silage. In particular, *L. diolivorans* is an efficient inoculant because it produces antibacterial peptides that prevent the increase of saprophyte in silage.

Keywords: Potato Pulp Silage, Inoculation, Lactic Acid Bacteria, *L. Lactis*, *L. Diolivorans*

Introduction

Potato pulp is a by-product of the starch industry, where processing can generate approximately 10% waste pulp (Oda et al., 2002). Potato production in China is the largest in the world, producing >20% of the global yields (Tian et al., 2017). Although potato pulp is suitable as a livestock feed because it comprises starch, peptic substances, and minerals (Mayer et al., 1997; Okine et al., 2005), the product decays easily when exposed to several pathogenic bacteria during preservation for high moisture content (Charmley et al., 2006; Tawila et al., 2008). Therefore, most of the potato pulp (5 million tons/year) is discarded as waste without effective utilization (NBSC 2010). The high energy costs associated with drying and processing the pulp are deterrent to its production and use. Therefore, effective and energy efficient means of processing are necessary for economic and environmental benefits (Tian et al., 2017). Ensiling is an efficient method to prevent potato pulp with high moisture content (McDonald 1991; Kayouli 1999) if the following three requirements are met: (1) adequate levels of fermentable substrate, (2) low buffering capacity, and (3) dry matter contents within 250–400 kg (Wilkinson

et al., 2005). Sugimoto reported that the addition of beet pulp, which is an agricultural by-product with very low moisture content, to potato pulp helped reduce the moisture content in the potato pulp silage (Sugimoto et al., 2010).

As an added benefit, inoculation of the pulp with lactic acid bacteria or other bacteria under anaerobic conditions improves the quality of silage and exerts probiotic effects that can enhance livestock performance (Daniel et al., 2018). The production of lactic acid reduces the pH of silage by approximately 4.0 (MacDonald et al., 2002). It has been previously reported that potato pulp ensiled with bacterial or fungal inoculants *L. rhamnosus* or *Rhizopus oryzae*, respectively, for 50 d showed increased levels of lactic acid and carbohydrates, whereas starch and pectin content decreased, suggesting that the modified potato pulp silage is a suitable energy source of ruminant feed (Aibibula et al., 2007). Moreover, Oda reported that *R. oryzae* IFO04707 decreased the pH of potato pulp within 1 d and then produced lactic acid under aerobic conditions (Oda et al., 2002). The highest concentration of lactic acid was observed after 6-d of fermentation. Amylases secreted from the fungus also hydrolyzed starch to produce a water-soluble carbohydrate. Therefore, fungus is an effective inoculant for use in ensiling of potato pulp.

L. lactis inoculation of grass silage reportedly increases lactic acid content and produces good quality livestock feed (Charmly et al., 1966), and the relationship between inoculation and livestock performance has been reported by several authors (Gallo et al., 2018; Li et al., 2018; Xu et al., 2018). Ellis also reported that the fermentation quality and nutritive value of grass silage was improved by the addition of *L. lactis* (Ellis et al., 2016). Previously, we have found that a lactic acid bacterium, *L. diolivorans*, isolated from Mongolian fermented mare milk, "airag", produces peptides with molecular weights of approximately 2000 Da and has potent antibacterial activity within a wide range of temperatures and pH (Oyundelger et al., 2016).

In this paper, we report the protein and lactic and acetic acid production in potato pulp inoculated with *L. lactis* and *L. diolivorans* to demonstrate the quality of the resultant potato silages.

Materials and Methods

Material

For analysis, potato pulp was dried for 48 h at 60°C and then for 4 h at 105°C. The dried potato pulp was ground to powder by passing through a 1-mm screen and then stored at room temperature until use. Lactic acid bacteria, *L. lactis* NCIMB 30160 and *L. diolivorans* SBS 0007 for inoculation were purchased from the Institute of Microbiology, Xinjang Academy of Sciences, Urumqi, China, and Snow Brand Seed Co. Ltd., Sapporo, Japan, respectively. Neutral detergent solution was prepared by mixing the following 4 reagents in 1 L of distilled water, ethylenediamine tereacetic acid disodium salt (18.6 g, 0.05 mol), sodium borate (6.8 g, 0.02 mol), sodium dodecyl sulfate (3.0 g, 0.01 mol), sodium phosphate (4.6 g, 0.03 mol), and triethylene glycol (6.8 g, 0.05 mol). The mixture was stirred for 1 h at 100°C to produce a clear solution. Ethyl trimethyl ammonium bromide (20 g, 0.05 mol) solution in 1N sulfuric acid (1 L) was used as the acid detergent solution.

Inoculation and Ensiling

Inoculation with *L. lactis* was as follows. Dry *L. lactis* (0.15 g, 1×10^8 cell/g) was dispersed in water (30 mL), and the mixture was then sprayed onto the fresh potato pulp (30 kg). The inoculated potato pulp was ensiled in a mini-silo under anaerobic conditions for 30 d at room temperature between 22°C and 25°C. Ensiling with *L. diolivorans* inoculation was performed in the same manner and conditions. Each experiment was performed three times, and the average values of the replicates were used in the analyses.

Component Analysis

The content of organic matter and ash was measured by heating ensiled pulp at 550°C for 4 h according to the method of AOAC (AOAC 1990). Protein and fat contents were determined by a Soxhlet extraction with hexane for 24 h, respectively, according to the AOAC method (AOAC 1990). Soluble sugar content was obtained from the dried potato pulp (2 g) by extraction with a mixture of ethanol (800 mL) and water (200 mL) at 80°C. This was repeated three times and the extracts were then hydrolyzed by 5% aqueous sulfuric acid. Starch in the dried potato pulp (2 g) was extracted for 2 h by boiling water with 60% of $HClO_4$ aqueous solution (600 mL), and the extracts were measured for starch content by a spectrophotometric method. Acetic, lactic, propionic, and butyric acid contents were measured by a HPLC according to the method of AOAC (AOAC1990). The neutral and acid detergent fiber (NDF and ADF, respectively) contents were analyzed according to the methods reported by Van Soest (Van Soest et al., 1991). The powdery, dried potato pulp (1 g) was added to 100 mL of the neutral detergent solution. This mixture was then boiled for 1 h, and the precipitate was collected by vacuum filtration using a glass filter (Whatman grade 4). The filtrate was washed with acetone (20 mL) and dried for 2 h at 105°C to yield 0.917 g of NDF. The ADF (0.650 g) was obtained by boiling the dried potato pulp (1.0 g) with the acid detergent solution (100 mL) for 1 h in the above-mentioned manner. Starch content in the dried potato pulp (1.0 g) was determined by boiling the mister with 60% $HClO_4$ aqueous solution (100 mL) for 2 h. Hemicellulose content was calculated from the difference between the NDF and ADF contents. The results of the component analysis were performed three times, and the average values were used.

Results and discussion

Inoculation of Potato Pulp with Lactic Acid Bacterium

Potato pulp itself is not suitable as a livestock feed because of its high moisture content (>800 g/kg) and low amount of protein. Ensiling with inoculant is an accepted method to increase nutritional value of crops. However, inoculation of potato pulp with *L. rhamnosus* or *R. oryzae* strains did not increase protein content (Okine et al., 2005). However, *L. diolivorans* showed potent antibacterial activity against *E. coli* and *B. subtilis* across a wide range of pH and temperature that appeared to originate from the produced peptides (Oyundelger et al., 2016). Therefore, *L. diolivorans* is a suitable lactic acid bacterium for use as an inoculant in potato pulp ensiling; it also reportedly prevents saprophyte propagation by the production of antibacterial peptides during fermentation. The produced peptides are also important for nutrition. *L. lactis*, a lactic acid bacterium produced in the

manufacture of fermentation foods has also been successfully used for the inoculation of potato pulp (Li et al., 2017).

Table 1 summarizes the results of the analysis of potato pulp ensiled in mini-silos for 30 d at room temperature under anaerobic conditions with *L. lactis* and *L. diolivorans* and then dried for 48 h at 60°C and then for 4 h at 105°C. The moisture content in the potato pulp remained unchanged at 822 g/kg before and after ensiling.

Table 1 Analysis of potato pulp after inoculation for 30 d at room temperature.[a, b]

	Organic substance							Ash
Inoculant	Total	NDF[c]	ADF[d]	Starch	Soluble sugar	Protein	Fat	
	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg
Potato pulp[e]	967	363	348	206	5	39	6	33
Control[f]	974	364	348	195	15	46	6	26
L.lactis[g]	975	360	345	196	10	57	7	25
L.diolivorans[g]	964	354	325	208	13	58	6	36

[a]Original potato pulp had 822 g/kg of moisture.

[b]Before analysis, potato pulp was dried for 48 h at 60°C and then for 4 h at 105°C.

[c]NDF: Neutral detergent fiber consisting mainly of cellulose, hemicellulose, and lignin.

[d]ADF: Acid detergent fiber consisting mainly of cellulose and lignin.

[e]Original potato pulp.

[f]Without inoculation.

[g]Inoculation with *L. lactis* and *L. diolivorans*, respectively.

Similarly, ensiling did not drastically affect the content of organic substances. The NDF obtained by treatment with the neutral detergent solution decreased only slightly from 363 g/kg before ensiling to 360 and 354 g/kg for *L. lactis* and *L. diolivorans* inoculations, respectively. The ADF obtained by treatment with the acidic detergent solution also only decreased to 345 and 325 g/kg after ensiling with *L. diolivorans* inoculation responsible for the decrease of the greatest decrease in ADF contents. Hemicellulose and starch were somewhat increased and decreased, respectively, before ensiling. The soluble sugar content increased after ensiling, the digestion of starch and hemicellulose. The protein content also increased to 57 and 58 g/kg due to peptide production by *L.*

lactis and *L. diolivorans* lactic acid bacteria, which worked more effective for the production of proteins than the previous reported *L. rhamnosus* and *R. oryzae* bacteria (Okine et al., 2005). The fat content in the potato pulp remained low even after ensiling. These results suggest that potato pulp ensiled with lactic acid bacteria improves the quality of potato pulp as a livestock feed.

Acid Content in Potato Pulp After Ensiling

Figure 1 presents the relationship between pH and lactic acid production during ensiling. The pH of the potato pulp silage decreased rapidly to approximately 4.0 after 10 d and then gradually decreased below 4.0 as the production of lactic acid increased. After ensiling for 30 d, lactic acid content was 52 and 50 g/kg for *L. lactis* and *L. diolivorans* inoculated silage, respectively. Moreover, *L. diolivorans* reportedly increases the quality of potato pulp silage by the production of antibacterial peptides (Oyundelger et al., 2016).

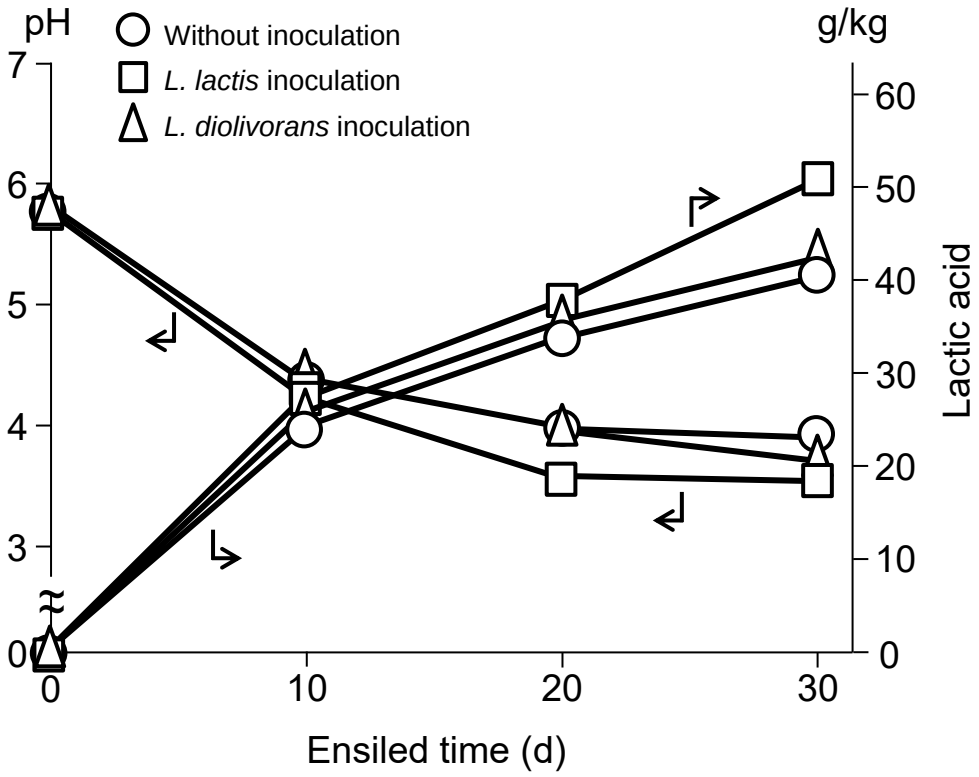

Fig. 1. Relationship between pH (left) and production of lactic acid (right) on potato pulp fermentation with lactic acid bacteria. Lactic acid content was measuredafter drying for 48 h at 60°C and subsequent for 4 h at 105°C.

Table 2. Acid content in potato pulp silage.[a]

Inoculant	Moisture	pH	Lactic acid	Acetic acid	Prop ionic acid	Butyric acid
	g/kg		g/kg	g/kg	g/kg	g/kg

Potato pulp[b]	822	5.8	2	0	0	nd
Control[c]	822	3.9	41	10	9	nd
L.lactis[d]	838	3.6	52	9	9	nd
L.diolivorans[d]	816	3.8	50	8	7	nd

[a]Acid content was measured after drying for 48 h at 60°C and subsequent for 4 h at 105°C.

[b]Original potato pulp had 822g/kg of moisture before ensiling.

[c]Without inoculation.

[d]Inoculation with *L. lactis* and *L .diolivorans*, respectively.

Table 2 summarizes the acid content in the potato pulp after ensiling. The acid content in the potato pulp before ensiling was low. After ensiling, lactic, acetic, and propionic acid contents increased, whereas toxic butyric acid was not detected. The control silage also produced similar concentration of organic acids possibly due to lactic acid bacteria contained in the pre-inoculated potato pulp or present in the mini-silo used for storage. However, inoculation with *L. lactis* and *L. diolivorans* yielded large amounts of lactic acid and peptides and decreased the pH below 4.0. These results indicate that the quality of potato pulp was improved by inoculation with *L. lactis* and *L. diolivorans*.

Conclusion

Ensiling of potato pulp with lactic acid bacteria *L. lactis* and *L. diolivorans* reduced the pH below 4.0, whereas lactic, acetic, and propionic acids were produced; however, no toxic butyric acid was detected. The protein content of inoculated silage increased due to the production of peptides by the lactic acid bacteria. The NDF content after ensiling decreased with a decrease in the ADF content. These results demonstrated that the inoculation of potato pulp with *L. lactis* or *L. diolivorans* is effective in improving the quality of the potato pulp silage. Notably, *L. diolivorans* lactic acid bacterium produced a large increase in peptide and lactic acid content; therefore, *L. diolivorans* is expected to be an efficient inoculant. The efficacy of *L. diolivorans* and other lactic acid bacteria in improving the yield of potato pulp ensiling needs further exploration to establish the usefulness of the pulp as livestock feed. In addition, we plan to further investigate the moisture content in potato pulp before and after ensiling with lactic acid bacteria.

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Conflict of interest

The authors have no conflict of interest to disclose with respect to this article.

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