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Jurnal Biota Vol. 11 No. 2 (2025) https://doi.org/10.19109/biota.v11i1.27177
Research Article

Nutritional Analysis of Different Maize Varieties and Silage Produced at Haor Area in Sylhet

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Received: 25/01/2025	Revised: 08/02/2025	Accepted: 12/03/2025	Online:12/03/2025

ABSTRACT
Maize and silage play a critical role in livestock nutrition, offering a cost-effective feed with a balanced nutrient profile. Improving maize and silage quality is essential for maximizing animal performance. This study aimed to evaluate the nutritional composition, fiber content, pH levels, and bacterial activity in silage made from two maize varieties—KMHB410 and HMS-PS-3355—using varying levels of molasses as an additive to improve silage quality. The study was conducted in Sylhet, Bangladesh where an absence of green grass causes the cattle to suffer from malnutrition throughout the lean season. Here silage was produced by mixing the chopped maize with 5% and 10% molasses, along with a control group. After 15 days of fermentation, the silage was assessed for dry matter (DM), ether extract (EE), crude protein (CP), crude fiber (CF), and acid detergent fiber (ADF), and the presence of Lactobacillus spp. The study of Dry matter (DM) content ranged from (8.54 to 17.25) %, with HMS-PS-3355 at 17.25% and KMHB 10% molasses at 8.54% (P=0.002). Crude protein (CP) varied significantly (P=0.002), with KMHB C showing the highest value at 19.04%, while HMSC recorded 10.36%. The addition of molasses significantly reduced acid detergent fiber (ADF) content. Bacterial colony-forming units (CFU) were highest in the control silage (97×10^6 CFU), while the 10% molasses treatment had the lowest count (38×10^6 CFU), indicating that increased molasses concentrations reduced microbial growth. Confirmation and screening of Lactobacillus spp. in silage was carried out by culturing the microorganisms in a lactobacillus selective MRS media followed by different biochemical tests.

Keywords: *Bacterial strain; Biochemical; Phylogenetic; Silage; Zea mays.*

Introduction

Since independence, Bangladesh's agriculture sector, which accounts for 15.89% of the nation's GDP, has undergone substantial changes. The shift from traditional farming to more modern methods has been largely influenced by the Green Revolution, which familiarized high-yield crop varieties, chemical fertilizers, and improved irrigation systems [1]. This transformation has boosted crop yields and contributed to national food security. However, it also presents challenges such as environmental harm, the need for sustainable farming practices, and ensuring that smallholder farmers benefit from these advancements [2].

Livestock production is a key element of Bangladesh's agricultural sector but its growth and productivity are hindered by several challenges. These include limited availability of feed resources, the poor nutritional quality of existing feed, widespread disease outbreaks, and the low genetic potential of livestock species, all of which contribute to low overall

productivity [3]. In tropical areas like Bangladesh, fodder yields are lower and there is often a shortage of available fodder during the summer and winter months [4]. Lean periods occur from October to December and April to June, during which fodder availability is limited. Fodder is generally abundant for the rest of the year. In terms of feed quality, silage is often regarded as superior to hay, as it requires less time to wilt the fodder, leading to a smaller decrease in its nutritional content [5].

The maize (*Zea mays* L.) is a crop first domesticated in Mesoamerica thousands of years ago. Today, it is a vital staple across the globe, playing a key role in both human and livestock nutrition. As Norman Borlaug once highlighted, through advancements like silage, maize contributes significantly to global food security and agricultural sustainability [6]. Producing silage from maize can effectively address the issue of fodder scarcity. Maize silage provides a steadily high feed price, boasts a high liveliness gratify, is highly palatable for livestock, and is also ecologically maintainable [7]. Following rice and wheat, maize is the third most significant cereal crop in Bangladesh, known for its considerable nutritional benefits. In terms of yield, maize has become the top cereal crop, producing an average of 6.15 tons per hectare, surpassing wheat, which yields about 2.60 tons per hectare, and boro rice, which yields approximately 3.90 tons per hectare [8], [9]. Hassan *et al* [10], examined the costs and profitability of maize production in Bangladesh and found that cultivating maize is advantageous. In a separate three-year trial, Assefa *et al* [11] evaluated rice, maize, mung bean, and sunflower in Bangladesh, revealing that maize generated the highest net income among the crops studied. Additionally, *Zea mays* have the potential to deliver substantial amounts of dynamism-rich silage for livestock, and it can be safely fed at a slight growth phase without the jeopardy of prussic acid oxalic or acid toxicity [12]. The most important prerequisite for silage production is anaerobic conditions because these bacteria convert sugars into lactic acid, a potent organic acid. Reduced pH inhibits the growth of spoiling bacteria and the degrading activities of plant enzymes and unwanted microorganisms. When processing maize to make silage, molasses is added sometimes. It serves as a feed additive and a great source of carbohydrates. It also supplies nutrients needed for the growth of desired (LAB) [13]. The utilization of maize silage could serve as an effective strategy for maintaining livestock production even under adverse climate conditions during the rainy season. In the present study, experimental plastic bag silage system production has been conducted on cultured maize land of the Dakshin Surma area, and its nutritional and microbial evaluation has also been carried out to ensure its quality as an alternative to forage during the lean period. The objectives of the experiment are the comparison of production and nutritional components between two maize varieties to ensure their quality and the comparison of assay of different silages using different levels of molasses in maize fodder.

Materials and Methods

Study materials and period

Two varieties of maize, KMHB410, and HMS-PS-3355 were selected for nutritional assessment during the 65-day growth stage in Hajiganj, Dakshin Surma, Sylhet. The study period was from November 2023 and April 2024. At this period, the maize was collected, and the efficiency per square meter was measured by weighing the yield. Additionally, the length of five randomly selected maize plants from each plot was recorded. The harvested maize was then transported to the Biochemistry laboratory of SAU for nutritional analysis. Subsequently, silage was prepared from the maize, incorporating two variants 5% and 10% molasses, and a

control batch with no molasses for each maize variety. After a 15-day fermentation period, the hay was collected and also sent to the Biochemistry laboratory of SAU for nutritional evaluation.

Proximate analysis

Proximate analysis of maize samples was approved using the measures provided in [14], [15].

Determination of dry matter and ash

In the laboratory, crucibles were utilized to store and analyze two grams of samples. The sample, along with crucibles was placed in an oven at 105°C overnight to dry and were then allowed to cool in a desiccator before being reweighed. To assess the ass content, the dried sample and crucibles were incinerated in a muffle furnace at 600°C for five hours. After cooling, the ash and crucibles were reweighed, enabling the calculation of dry matter and ash percentages using specific equations [15].

Determination of ether extract

A 2-gram sample was placed in a thimble for ether extraction using the Soxhlet apparatus. The thimble was inserted into the Soxhlet extractor, which was connected to a boiling flask. Diethyl ether (150 ml) was introduced through the top of the apparatus. The sample solution was heated until it became clear, and then it was separated from the boiling flask. After cooling, the flask was weighed to complete the extraction process [15].

Determination of acid detergent fiber

A 1-gram sample was placed in a beaker, and 100 ml of ADS was added. The combination was boiled for one hour before being filtered. The residue was eroded with hot water and then treated with acetone. Afterward, it was dried at 105°C for 8 hours. Once cooled, the sample and crucible were weighed. The residue was then incinerated at 600°C for 2 hours, after which the ash was weighed [15].

Determination of crude fiber

A two-gram example was weighed and transferred into a conical bottle. To this, 200 ml of 0.128M H_2SO_4 was added, and the mixture was boiled for 30 minutes. After filtration, 200 ml of 0.313M NaOH solution was added to the flask. The filtrate was collected in a clean crucible and placed in a warm air oven at 230°C for 2 hours. Afterward, the pot containing the fiber was weighed, and the weight was recorded. The fiber was then incinerated in a quiet furnace at 550°C for 2 hours. Once airconditioned in a desiccator, the ash-filled crucible was reweighed, and the final weight was noted [15].

Determination of nitrogen-free extract

The nitrogen-free extract (NFE) is the only component of the proximate analysis that is estimated by calculation rather than chemical analysis. It is determined by subtracting the percentages of crude protein, ether extract, crude fiber, and ash content from 100, followed by appropriate calculations to record the NFE value.

$$NFE\% = 100 - (EE + CP + Ash + CF) \ldots (eq.1)$$

Silage production from maize

In this research, "Polythene Silage" was employed to harvest silage from maize in Dakshin Surma. Chopped maize, weighing 1 kg and cut into lengths of 1-3 inches, was mixed distinctly with 100 grams and 50 grams of molasses in polythene bags. The maize used was 65 days old and contained 65-70% moisture, which is optimal for silage production. The mixture was compressed to eliminate air.

pH measurement of silage

To assess the pH of the silage, a fresh sample that was 15 days old was collected. A 150 ml beaker was filled halfway with the silage, and enough water was added to cover the sample, leaving approximately 1/2 inch of free water at the

top. This mixture was permissible to stand for 30 minutes. The water was then drained from the silage into a separate beaker. Using a calibrated pH meter along with buffer solutions of pH 4.0 and 7.0, the pH of the solution was measured immediately [16].

Isolation and Identification of Lactic Acid Bacteria (Lab) From Silage

Cultivation on MRS Medium

Microbes from the silage were initially refined in nutrient broth and after twenty-four hours of cultivation, these microorganisms were transported to *lactobacillus*-specific MRS media. After three days of vaccination, whitish round colonies emerged, which were then subcultured for additional analysis.

Biochemical testing for Confirmation of LAB from Silage

To confirm the presence of *Lactobacillus spp*. Several biochemical tests were conducted including Gram Staining, catalase test, oxidase test, indole test, methyl red (MR) test, Voges Proskauer (VP) test, and carbohydrate fermentation test.

Molecular Identification of Lactobacillus from Maize

Bacterial genomic DNA isolation protocol

The procedures at the National Institute of Biotechnology lab were followed to formulate DNA from bacterial colonies. The bacterial colony was inoculated into nourishment broth and educated rapidly at 37°C, after which it was transferred to an Eppendorf tube. The tube was centrifuged, and a lysis buffer containing proteinase K and RNAse A was added. A mixture of phenol, chloroform, and isoamyl alcohol was then introduced, followed by centrifugation. The aqueous layer was carefully transferred to a new tube. DNA was precipitated using ethanol, centrifuged, air-dried, and subsequently dissolved in TE buffer. The extracted DNA was amplified through PCR. The PCR products were analyzed using the dideoxy chain termination method on a Sanger machine at Wuhan Tianyi Huayu Gene Technology Co., Ltd [17].

Phylogenetic tree analysis

Molecular Evolutionary Genetics Analysis (MEGA) is a software tool designed for analyzing molecular evolution and constructing phylogenetic trees. These trees provide graphical representations of evolutionary relationships and similarities [18]. In a phylogenetic tree, each leaf node represents a species, while the edges illustrate the relationships between them, with edge lengths indicating the evolutionary distance. MEGA employs the neighbor-joining (NJ) clustering method for analysis. Bootstrap values above 70 are considered "well-supported," while those ranging from 50 to 70 are regarded as "moderately supported" [19].

Results and Discussion

Comparison of Nutrient Composition Among KMHB and HMS Feed Samples

In the results, the proximate composition and fiber content of three samples (KMHB 1, KMHB 2, and HMS) were analyzed and compared (Table 1). The DM (%) ranged from 13.39% to 17.25%, with HMS showing the highest value (17.25%). The ash content (%) varied between 0.87% and 1.05%, with no significant differences among the samples (P=0.412). EE (%) ranged from 3.82% to 5.82%, with HMS having a slightly higher fat content (P=0.425). CP (%) ranged from 8.21% to 12.33%, with KMHB 2 having the highest protein content. CF (%) and ADF also varied, with KMHB 1 showing the highest values for both (23.48% CF and 47.37% ADF). However, the nitrogen-free extract (%) showed a significant difference (P=0.026), with HMS having the highest carbohydrate content (66.69%). These findings were similar to those of Kennedy *et al* [20] as well as the comparison of nutrient composition in the feed silage. Debnath *et al* [21] reported comparable

findings regarding ADF, with the laboratory analysis showing an ADF of 31.048%. The higher ADF, which was indicative of lignocellulosic fiber, was often linked to reduced digestibility, as ADF is less readily degraded by rumen microorganisms [22]. These findings are consistent with the results reported by Li and Wu [23], both of which found that variations in feed composition, including differences in fiber, protein, and carbohydrate content, are common across different feed types.

Table 1. Comparison of Nutrient Composition Among KMHB and HMS Feed Samples

Sample ID	DM%	Ash%	EE %	CP %	CF %	ADF	NFE
KMHB 1	16.30ab	1.05abc	3.82abc	8.85c	23.48c	47.37	63.01ab
KMHB 2	13.39abc	0.88bc	4.82abc	12.33abc	22.96c	46.12	59.63b
HMS	17.25a	0.87bc	5.82bc	8.21c	20.95cd	41.84	66.69a
P-Value	0.289	0.412	0.425	0.334	0.279	0.648	.026

Nutrient Composition and pH Levels in KMHB and HMS Feed Samples at Varying Concentrations

In the results, significant variations were observed in the proximate composition, fiber content, and pH across the different treatments of KMHB and HMS at 5% and 10% inclusion levels, along with their KMHB C and HMS C groups (Table 2).

Table 2. Nutrient Composition and pH Levels in KMHB and HMS Feed Samples at Varying Concentrations

Sample ID	DM%	Ash%	EE %	CP %	CF %	ADF	NFE	pH
KMHB 5%	10.3bc	0.99abc	6.82a	16.38ab	30.48ab	48.41bc	59.42ab	4.19bc
HMS 5%	12.92abc	1.08abc	9.82abc	13.40abc	27.54bc	47.07c	57.86ab	3.94c
KMHB 10%	14.19ab	1.24ab	7.82abc	10.85bc	15.32cd	42.84d	73.08a	3.92ab
HMS 10%	15.21ab	1.37a	10.82abc	10.36bc	23.80bc	45.92cd	60.10ab	4.82c
KMHB C	8.54c	0.73c	8.82ab	19.04a	34.67a	52.71a	52.23b	4.66a
HMS C	15.88ab	1.36a	11.82c	10.47bc	24.57c	50.35ab	57.83ab	5.12a
P-Value	0.002	0.001	0.015	0.002	0.001	.000	0.026	0.002

DM varied significantly (P=0.002), ranging from 8.54% to 15.88%, with HMS C showing the highest DM content at 15.88%. Ash content (%) also exhibited significant variation (P=0.001), with HMS 10% and HMS C having the highest ash values, indicating increased mineral content in these samples. Ether Extract (%) showed significant differences (P=0.015), with HMS C containing the highest fat content (11.82%), highlighting its richer lipid profile. CP was significantly different among samples (P=0.002), ranging from 10.36% to 19.04%. KMHB C had the highest protein content at 19.04%, indicating its superior protein composition. Both CF (%) and ADF differed significantly across the samples, with KMHB C having the highest fiber content (34.67% CF and 52.71% ADF) (P=0.001 and P=0.000, respectively), indicating its higher indigestible fiber fraction. Nitrogen-Free Extract (%) showed significant variation (P=0.026), with KMHB 10% containing the highest carbohydrate content (73.08%), reflecting its higher energy potential. pH values were significantly different (P=0.002), with HMS C having

the highest pH at 5.12, while HMS 5% exhibited the lowest pH at 3.94, indicating variation in acidity across the samples. A higher DM content, such as that observed HMS C, typing indicates better feed preservation and reduced moisture. It might contribute to enhanced nutrient concentration and longer shelf life [24]. This study also reported comparable findings regarding pH ranging from 3.97 to 3.66 in maize silage. This is particularly important for livestock nutrition, as minerals play crucial roles in bone formation, metabolism, and overall health [25]. The increased mineral content in these samples could provide better mineral nutrition for animals, potentially improving performance in production systems that rely on mineral supplementation.

Comparison of Plant Height and Weight Between KMHB and HMS Samples

In the results, the height and weight of the samples KMHB 1, KMHB 2, and HMS were compared. While the height did not show significant differences among the samples (P=0.412), with values ranging from 140.15 cm (HMS) to 156.00 cm (KMHB 2), there was a statistically significant difference in weight (P=0.001). KMHB 2 had the highest weight (4.115 kg), while HMS had the lowest weight (2.685 kg). This indicates that weight differences between the samples were significant, whereas height remained consistent across the groups (Table 3). These findings align with the results of Han *et al* [26], who also reported minimal variation in plant height in maize fields, with heights ranging from 290.0 to 291.7 cm. Similar to this study, Han *et al* [26] concluded that factors such as genotype or cultivation practices had a negligible impact on height when controlled for other variables. In this context, it is plausible that the inherent genetic factors or other management conditions (e.g., irrigation, sunlight, etc.) may have exerted minimal influence on plant height across the different samples, leading to a relatively uniform outcome.

Table 3. Comparison of Plant Height and Weight Between KMHB and HMS Samples

Sample ID	Height (cm)	Weight (kg)
KMHB 1	152.500	4.040
KMHB 2	156.000	4.115
HMS	140.150	2.685
P-Value	0.412	0.001

Effect of Molasses Concentration on Nutrient Composition and pH Levels

The analysis of DM (%), Ash (%), EE (%), CF (%), and ADF revealed trends, although their P-values were not statistically significant (P>0.05) except for ADF. These results suggest that increasing molasses concentrations significantly influenced protein levels, fiber digestibility, carbohydrate content, and acidity (Table 4).

Table 4. Effect of Molasses Concentration on Nutrient Composition and pH Levels

Conc. of molasses	DM%	Ash%	EE %	CP %	CF %	ADF	NFE	pH
0	12.21a	1.04a	5.12a	14.76a	29.04a	51.53a	49.20b	5.03a
5%	11.64a	1.03a	4.33a	14.89a	28.75a	47.74b	48.87b	4.30b
10%	14.70a	1.30a	3.85a	10.61b	19.76a	44.38b	65.55a	4.05b
P-Value	0.097	0.085	0.079	0.049	0.066	0.001	0.021	0.002

Crude protein (%) was significantly affected by molasses concentration (P=0.049). The highest CP was observed at 0% and 5% molasses (14.76% and 14.89%,

respectively), while the 10% molasses treatment had a significantly lower CP (10.61%). ADF showed a significant reduction (P=0.001) as molasses concentration increased, with the highest value at 0% molasses (51.53%) and the lowest at 10% molasses (44.38%), indicating improved fiber digestibility with higher molasses levels. NFE significantly increased with 10% molasses (65.55%) compared to lower concentrations (P=0.021), reflecting higher carbohydrate content at this level. pH values were significantly different (P=0.002), with the highest pH at 0% molasses (5.03) and the lowest at 10% molasses (4.05), indicating increased acidity with higher molasses inclusion. This decrease in CP with increasing molasses inclusion is consistent with previous studies that found an inverse relationship between carbohydrate-rich additives, such as molasses, and protein content in feeds [27]. Molasses is a high-energy carbohydrate source, and its inclusion in the diet may result in a dilution effect on protein content. This effect could be due to the higher energy concentration in molasses potentially limiting the inclusion of protein-rich ingredients in the feed formulation. Moreover, the lower CP at higher molasses levels may affect the overall protein availability for livestock, particularly for growth and lactation, where higher protein intake is required [28].

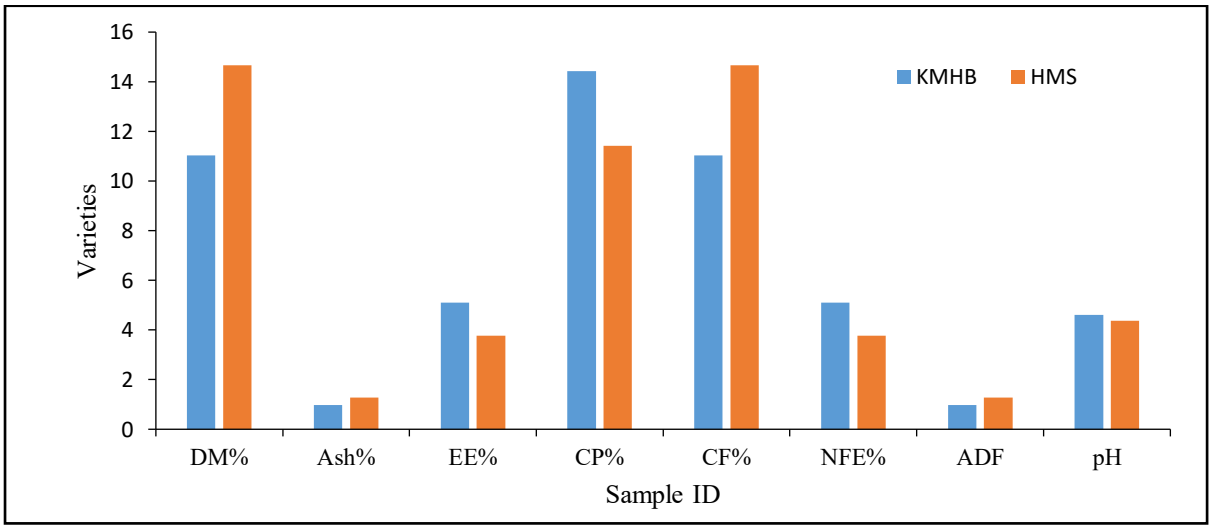


Figure 1. Nutrient Composition Comparison Between KMHB and HMS Feeding Regimens.

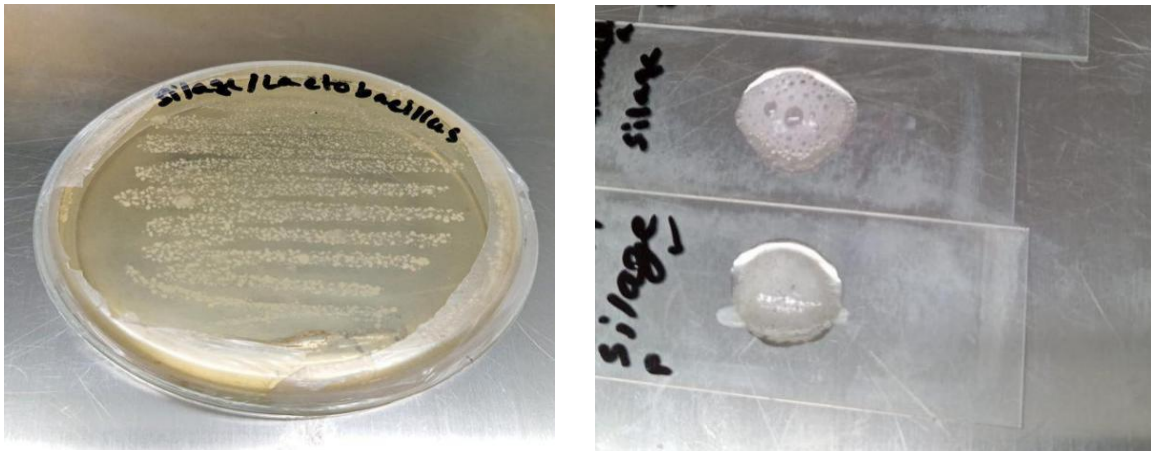
Nutrient Composition Comparison Between KMHB and HMS Feeding Regimens

The analysis of the nutrient composition of the feed samples revealed significant differences between the KMHB and HMS feeding regimens in several key parameters (Figure 1). The dry matter (%) content was significantly higher in the HMS group (14.67%) compared to the KMHB group (11.03%), P=0.009. Similarly, the ash content was also significantly greater in HMS (1.27%) than in KMHB (0.98%), P=0.017. The ether extract (%) showed a significant difference as well, with KMHB having a higher percentage (5.11%) compared to HMS (3.76%) P=009. Additionally, crude protein (%) levels were significantly higher in the KMHB group (14.42%) than in HMS (11.41%), P=0.017. However, no significant differences were observed in CF (%) between the two groups (11.03% for KMHB and 14.67% for HMS, P=0.544), nor in NFE (%) (5.11% for KMHB and 3.76% for HMS, p = 0.357). ADF also showed no significant variation, with both groups having similar values (0.98% for KMHB and 1.27% for HMS, P=0.830). The pH levels were comparable between the two regimens (4.60 for KMHB and 4.38 for HMS, P=0.118). Ash content

primarily reflects the mineral content of the feed, and higher ash values in HMS suggest that this feed may be richer in essential minerals such as calcium, magnesium, and phosphorus, which are vital for bone health and metabolic functions in livestock [29]. The increase in mineral content in HMS could have implications for livestock health, particularly in meeting their daily mineral requirements. However, excessive mineral content can also negatively affect the bioavailability of other nutrients, so a balanced mineral composition is crucial [27].

Screening of Lactobacillus spp.

For screening Lactobacillus spp., microbes from silage were cultured in nutrient broth at 37°C for 24 hours. The turbid brownish color of the broth indicated microbial presence. After 2 days on MRS media, bacterial culture appeared as small, white creamy colonies, indicating *Lactobacillus* spp. (Figure 2).



A B

Figure 2. Growth of bacterial colony in A. *lactobacillus* specific MRS media, B Catalyse test.

Table 5. Biochemical and Growth Characteristics of the Isolated Bacterial Strain

Items	Result
Biochemical test	
Gram staining	Gram-positive rods
Catalase test	Negative
Oxidase test	Negative
Indole test	Negative
Growth at different temperature	
15^0 C	No
37^0 C	Yes
50^0 C	Yes

Biochemical and Growth Characteristics of the Isolated Bacterial Strain

The biochemical tests and growth characteristics of the isolated bacterial strain are summarized in Table 5. Gram staining revealed that the bacteria are Gram-positive rods. The strain tested negative for both catalase and oxidase activity, as well as for the indole test. The strain's ability to grow at different temperatures was also assessed. No growth was observed at 15°C, while the strain grew successfully at both 37°C and 50°C. These findings suggest that the bacterial strain is likely a thermotolerant organism, capable of growth at higher temperatures, typical of some Gram-positive species. These results were consistent with those of Chakra *et al* [30], whose study focused on isolating and biochemically characterizing plant growth-promoting bacteria from a maize field. The Gram-positive nature of the bacterial strain, indicated by the Gram staining, is a notable characteristic. Gram-positive bacteria are well known for their thicker peptidoglycan cell walls, which can confer resistance to certain environmental stresses and

antibiotics [31]. The absence of catalase and oxidase activity, alongside a negative indole test, suggests that the bacterial strain is not involved in specific enzymatic pathways commonly associated with oxidative stress resistance or tryptophan metabolism. These results are consistent with other studies that have identified Gram-positive bacteria lacking these activities [32], indicating that the strain may rely on other mechanisms to thrive in its environment.

Antibiotic Sensitivity Test

Antibiotic Sensitivity Profile of Isolated Bacterial Strain

In the results, the efficacy of three antibiotics Azithromycin, Ampicillin, and Tetracycline was tested against a microbial strain (Table 6). Azithromycin (30 µg) produced an inhibition zone of 15 mm, which is classified as intermediate (I), indicating moderate effectiveness against the organism. Ampicillin (30 µg) resulted in a 10 mm inhibition zone, categorized as resistant (R), signifying that the tested organism was resistant to this antibiotic. Tetracycline (30 µg) also showed a 15 mm inhibition zone, which, like Azithromycin, was classified as intermediate (I), reflecting moderate antimicrobial activity. These findings demonstrate that the organism exhibited resistance to Ampicillin, while Azithromycin and Tetracycline had intermediate effectiveness. Azithromycin, with a 15 mm inhibition zone, demonstrated an intermediate level of effectiveness (I) against the microbial strain. Azithromycin, a macrolide antibiotic, is commonly used for its broad-spectrum activity against both Gram-positive and Gram-negative bacteria [33]. The intermediate response in this study suggests that while the antibiotic is effective to a certain extent, higher concentrations or alternative therapies may be necessary to achieve complete inhibition. This finding aligns with previous studies that report varying levels of susceptibility to Azithromycin, with resistance or intermediate efficacy often noted in certain bacterial strains [34].

Table 6. Antibiotic Sensitivity Profile of Isolated Bacterial Strain

Antibiotics	Concentration	Range
Azithromycin	30 µg	15 mm (I)
Ampicillin	30 µg	10 mm (R)
Tetracycline	30 µg	15mm (I)

Bacterial colony count

Effect of Molasses Concentration on Bacterial Colony Forming Units (CFU)

The Table presents the bacterial colony count in different concentrations of molasses of KMHB silage. Higher bacterial growth was observed in the silage control compared to samples with 5% and 10% molasses (Table 7). In the results, the concentration of molasses had a notable impact on colony-forming units (C.F.U.). The control (0% molasses) had the highest microbial count, with 97×10^6 C.F.U. The 5% molasses treatment showed a reduction in the microbial count to 79×10^6 C.F.U. The 10% molasses treatment had the lowest microbial count, with 38×10^6 C.F.U. This suggests that increasing molasses concentration led to a decrease in microbial activity, with the control exhibiting the highest microbial growth and 10% molasses showing a significant reduction in C.F.U. These results were consistent with those of Chakra *et al* [30], whose study focused on isolating and biochemically characterizing plant growth-promoting bacteria from a maize field. The reduction in bacterial growth with increasing molasses concentration could be attributed to the higher sugar content in the molasses, which may alter the osmotic balance in the microbial environment. Higher concentrations of sugars can result in osmotic stress, which may inhibit the growth of certain bacterial species, especially those that are not adapted to high-sugar environments [35].

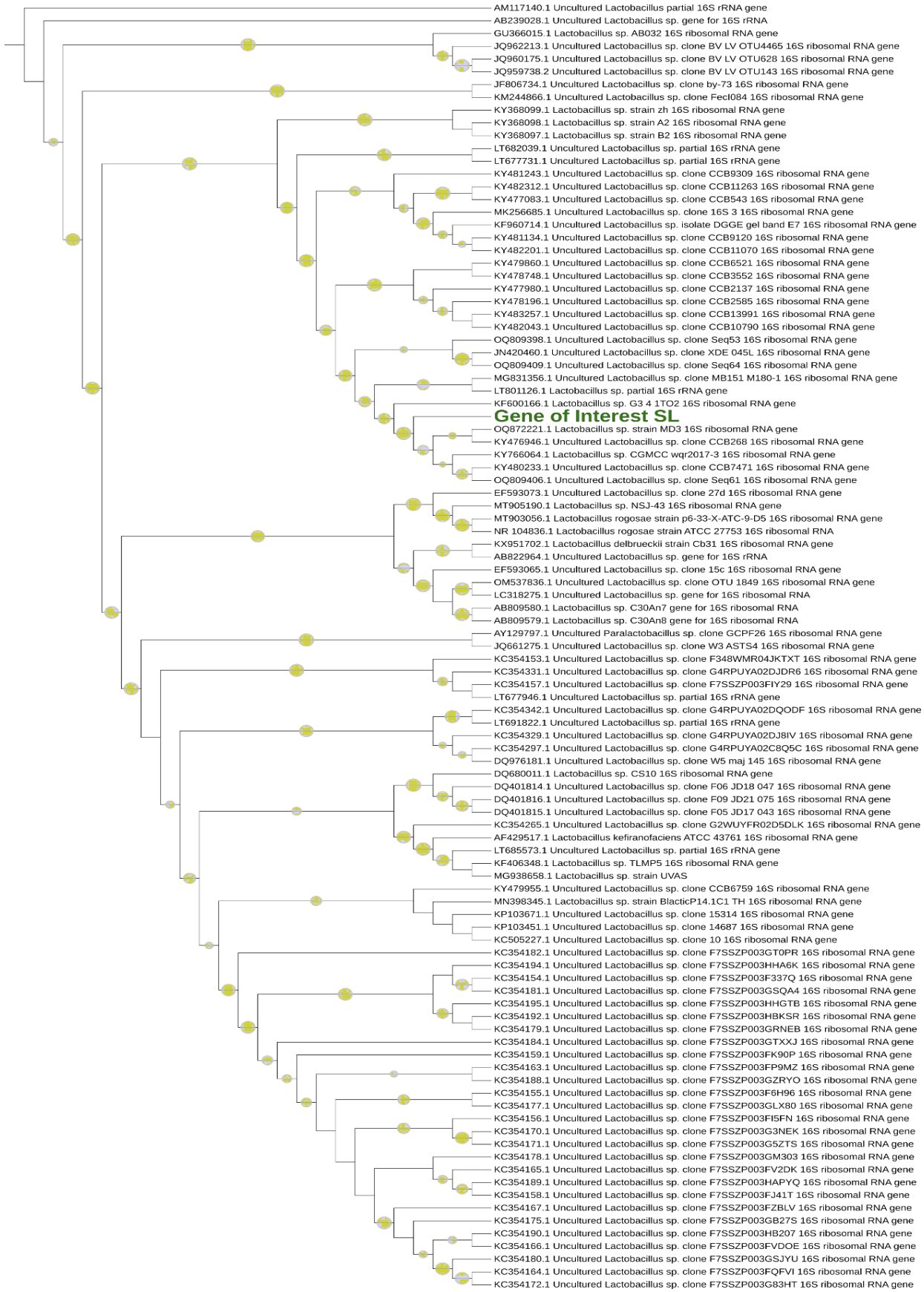


Figure 4. Representation of phylogenetic tree with highly similar sequences of Silage from maize isolate from NCBI. The green label is Gene of interest and the bootstrap value is 0.99. The highest bootstrap value is 1.

Table 7. Effect of Molasses Concentration on Bacterial Colony Forming Units (CFU)

Concentration of molasses	CFU
5%	79×106
10%	38×106
Control	97×106

Confirming Lactobacillus from Maize

The evolutionary history was determined using the Neighbor-Joining method by Saitou and Nei [36], and the resulting optimal phylogenetic tree is noted in Figure 3. The tree is scaled with branch lengths represented in the same units as the evolutionary distances utilized to generate the phylogeny (Figure 4).

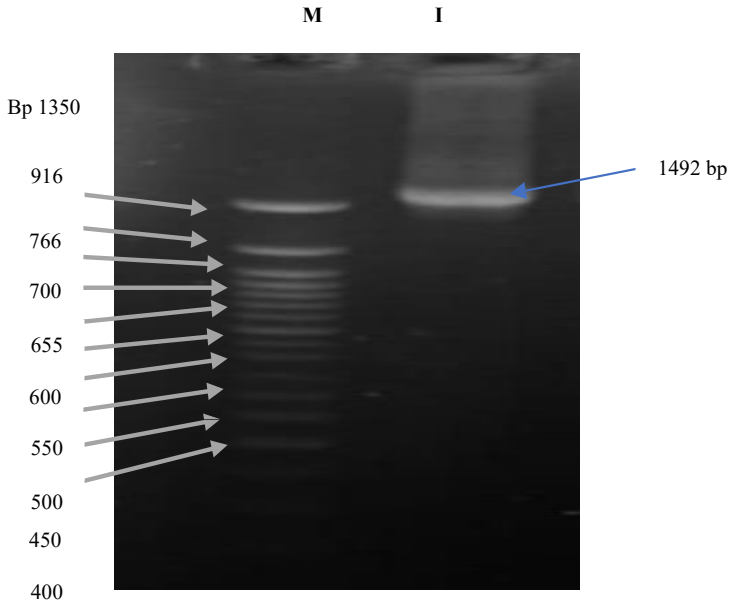


Figure 3. 16s rRNA region from maize silage bacteria isolates were amplified by PCR using primer names 27F and 149R and the products were separated by agarose gel electrophoresis, M-ladder; lane-1 for silage.

The evolutionary distances were calculated using the Maximum Composite Likelihood method Tamura *et al* [37], expressed as the number of base substitutions per site. The analysis involved 100 nucleotide sequences, with ambiguous positions being excluded for each pair of sequences (pairwise deletion option). In total, the final dataset comprised 1613 positions. All evolutionary analyses were performed using MEGA11 Tamura *et al* [38]. The bootstrap value of 99% indicates a high level of confidence in the result, demonstrating a 93% similarity of KF600166.1 *Lactobacillus spp.* G3 4 1TO2 16S ribosomal RNA gene and the sequence with an E value of 0. This confirms the presence of *Lactobacillus spp*. In the BLAST result, hits are automatically sorted by E-value, with the best hit displayed at the top. A lower E-value signifies a stronger match, with values less than 1e-50 considered an extremely high-quality match. BLAST hits with an E-value below 0.01 are still considered good for homology searches. The results of the evolutionary distance analysis, performed using the Maximum Composite Likelihood (MCL) method [6], [39], [40], provided important insights into the phylogenetic relationship of the KF600166.1 *Lactobacillus* spp. 16S ribosomal RNA (rRNA) gene sequence.

Conclusion

In the study, the nutrient composition, fiber content, pH levels, and

bacterial growth were analyzed across KMHB and HMS feed samples. KMHB showed higher protein and fiber content, while HMS had greater dry matter, ash, and carbohydrate content. Significant variations were observed in DM, EE, CP, CF, and ADF across treatments with different molasses concentrations, with increased molasses improving fiber digestibility but lowering microbial activity. The bacterial strain isolated was identified as thermotolerant, Gram-positive, and according to the DNA sequencing result we ensured that it was a *Lactobacillus* spp. and it was resistant to Ampicillin, with intermediate sensitivity to Azithromycin and Tetracycline. From DNA sequencing we ensured that it was a Lactobacillus spp. Molasses concentration notably impacted bacterial colony growth, with higher molasses reducing microbial counts.

Acknowledgments

The author would like to sincerely thank Mohammed Mehedi Hasan Khan, whose advice and ideas significantly expanded the scope of our study. We appreciate the financial assistance provided by KRISHI GOBESHONA FOUNDATION. Lastly, the authors express their gratitude to Sahil Abrar for their helpful criticism and technical support of the article.

Conflict of interest

We declare that there is no conflict of interest.

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