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CHARACTERISATION OF *Leuconostoc* spp. ISOLATES CAUSING SUGAR CANE JUICE QUALITY DETERIORATION IN KENYA

CARACTÉRISATION DES ISOLATS DE *Leuconostoc* spp. À L'ORIGINE DE LA DÉGRADATION DE LA QUALITÉ DU JUS DE CANNE À SUCRE AU KENYA

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ABSTRACT

Sugar cane (*Saccharum officinarum* L.) is one of several perishable industrial crops that should be processed immediately after harvesting. In Kenya, harvested sugar cane takes 1-7 days before milling. Delay beyond this period causes the sugar cane to deteriorate due to the *Leuconostoc mesenteroides* bacteria, which invade the harvested sugar cane through the cut ends. The objective of this study was to isolate and characterise isolates of *Leuconostoc* spp. from sugar cane-growing regions in Kenya. The study was conducted on isolates sourced from three main sugar cane growing regions in Kenya, namely Kakamega, Nyando and South Nyanza (Sony sugar). Four sugar cane varieties, namely KEN 83-737, KEN 82-808, N14, and Co421 were used for this purpose. The isolates were identified by their morphological and biochemical characteristics. Deterioration parameters (brix% juice, pol% juice, dextran and pH) were evaluated over 10 days. The results revealed that Sony sugar isolate was responsible for the highest levels of sugarcane deterioration, whereas the Kakamega and Nyando isolates exhibited the lowest levels of deterioration. N14 and Co421 were the most and least degraded sugarcane varieties, losing 2195.38 and 1093.16 g of sucrose to dextran, respectively.

Key Words: Brix% juice, Dextran, Pol% juice, *Saccharum officinarum*, sucrose

RÉSUMÉ

La canne à sucre (*Saccharum officinarum* L.) est l'une des nombreuses cultures industrielles périssables qui doivent être transformées immédiatement après la récolte. Au Kenya, la canne à sucre récoltée nécessite un délai de 1 à 7 jours avant d'être moulue. Au-delà de ce délai, la canne à sucre se détériore

en raison de la bactérie *Leuconostoc mesenteroides*, qui envahit la canne à sucre récoltée par les extrémités coupées. L'objectif de cette étude était d'isoler et de caractériser des isolats de *Leuconostoc* spp. provenant de régions productrices de canne à sucre au Kenya. L'étude a été menée sur des isolats provenant de trois principales régions productrices de canne à sucre au Kenya, à savoir Kakamega, Nyando et Nyanza Sud (Sony sugar). Quatre variétés de canne à sucre, à savoir KEN 83-737, KEN 82-808, N14 et Co421, ont été utilisées à cette fin. Les isolats ont été identifiés par leurs caractéristiques morphologiques et biochimiques. Les paramètres de dégradation (% Brix du jus, % Pol du jus, dextrane et pH) ont été évalués sur 10 jours. Les résultats ont révélé que l'isolat de sucre Sony était responsable des niveaux de dégradation les plus élevés de la canne à sucre, tandis que les isolats Kakamega et Nyando présentaient les niveaux de dégradation les plus faibles. N14 et Co421 étaient les variétés de canne à sucre les plus et les moins dégradées, perdant respectivement 2 195,38 et 1 093,16 g de saccharose au profit du dextrane.

Mots Clés: % Brix du jus, dextrane, % Pol du jus, *Saccharum officinarum*, saccharose

INTRODUCTION

Postharvest sugar cane (*Saccharum officinarum* L.) quality deterioration, attributed mainly to *Leuconostoc mesenteroides,* is a major hindrance to the prosperity of the sugar cane industry in sub-Saharan Africa (SSA). *Leuconostoc mesenteroides* is a bacterium that thrives on harvested sugar cane, thus converting sucrose into dextran, a polysaccharide which promotes juice viscosity and blocks crystallsation of sugar, causing significantly poor sugar yield (Asatkar and Basak, 2023; Candeliere *et al.*, 2024).

Sub-Saharan Africa is known for both production and consumption of cane sugar, in spite of the losses it incurs in postharvest quality deterioration (Kombo, 2023). Empirical data reveal substantial losses, with estimates ranging from 10 to 60%, depending on the nature of the crop and storage conditions. The Kenyan sugar cane sector has been a victim of this vice, with minimal successful strategies aimed at saving the industry (Kombo, 2023). In fact, there has been limited research on the primary varieties cultivated in the country, directly related to sucrose deterioration. In spite of the liberal access to numerous sugarcane varieties, research has not backed up the sector with working knowledge on the extent of the inherent sugar cane varietal predisposure to postharvest quality deterioration. It is imperative to leverage this genetic capacity to optimise quality conservation using minimal, inexpensive, and safe techniques. The objective of this study was, therefore, to isolate and characterise the major *Leuconostoc mesenteroides* bacteria from sugar cane growing regions in Kenya.

MATERIALS AND METHODS

Experimental site. A laboratory and field experiment was conducted at the Kenya Agricultural and Livestock Research Organisation (KALRO), Sugar Research Institute, Kibos, situated 1,250 meters above sea level at 0° 2'1" S and 34° 49'17" E.

Collection of *L. mesenteroides* isolates. Three *Leuconostoc mesenteroides* isolates were collected from three sugarcane-growing regions in Kenya (Kakamega, Nyando and Sony sugar) by harvesting three stalks from each of the six fields per region. This resulted in a total of 18 stalks per growing region. The stalks were chopped into halves and left overnight in the field to collect the bacteria. The chopped cane was then transported to the Sugar Research Institute pathology laboratory, where it was milled and the juice collected in 18 different containers and labeled accordingly for an overnight incubation.

Specific media for isolating *Leuconostoc mesenteroides* bacteria were prepared using the procedure described by Sarwat *et al.* (2008). The sample juice was inoculated using the sterile wire loop and incubated at 37°C for 48 hours, to allow colonies to develop.

Identification of *L. mesenteroides* isolates. The identification of these bacteria involved examining their morphological and biochemical traits. Morphological characteristics were evaluated based on the colony appearance on the media and through Gram staining, following standard protocols generated by Mujnisa and Natsir (2021). Biochemical characteristics included conducting carbohydrate fermentation and catalase tests (Khushboo *et al.*, 2023). After identifying the 18 individual samples, all the samples from each region were well-mixed to form a composite isolate, which was used to determine the deterioration levels on the selected sugar cane varieties (Table 1).

A field experiment was also conducted at KALRO Sugar Research Institute. The Institute's mean annual temperature is 19.7 °C, with mean annual precipitation around 1,912 mm. The soils consist mainly of heavy black clay (vertisols). The study area experiences a bimodal rainfall pattern, featuring two distinct rainy seasons each year: the long rains from March to May and the short rains from September to October. This rainfall pattern is typical of lake regions in Kenya (Evans e*t al*., 2020).

Experimental materials. The materials for the investigation included four sugar cane varieties commonly used in Kenya, according to the Cane Census report by the Sugar Directorate (2022). These were KEN 83-737, KEN 82-808, N14, and Co421; with respective area scale proportions of 30.04, 15.46, 2.51, and 36.58%, respectively (Ochami and Ochieng, 2020). Approximately 600 stalks of each variety were harvested and used during storage periods to assess the deterioration level of each variety by the three isolates. Three *Leuconostoc* spp. isolates were previously identified as described above.

Experimental design. The experiment was a 6 × 4 × 4 factorial, arranged in a randomised complete block design (RCBD). The factors included time at six levels connoting storage durations, namely P1 = 24, P2 = 48, P3 = 96, P4 = 144, P5 = 192, and P6 = 240 hours. The second factor was sugar cane variety at four levels, namely, KEN83-737, KEN 82-808, N14, and Co421. The third factor consisted of isolates at four levels, namely Kakamega isolate, Nyando isolate, Sony Sugar isolate, and a control. This resulted in 96 treatment combinations, replicated three times, totaling 288 experimental units.

The sugar cane varieties used in this study represented the commonly grown commercial genotypes in Kenya. Each stalk per variety was cut to 60 cm from the lower end of the stalk. Each variety had four bundles of 100 stalks, with an average weight of 10±0.85 kg. The sugar cane bundles were inoculated with the *Leuconostoc* spp. identified above. The inoculations were performed aseptically, using 1000 ml of 10^4 cfu per ml of each isolate, with one left un-inoculated as a control (Stern and Pretanik, 2006). After inoculation, each bundle was placed in a separate clean plastic container that was covered and labeled. Approximately 10 sets of cane were randomly selected in an aseptic manner from each container, crushed using the cane mill at the Sugar Research Institute (SRI), and the juice was collected in a clean sterile container for analysis.

Data collection. The quality parameters measured on the juice included brix% juice, pol% juice, dextran and pH. The % brix was measured by a refractometer (KRUSS OPTRONIC GERMAN), while the pol% juice was estimated by polarimetry (Anton Paar, saccharimeter). Dextran was evaluated by the

TABLE 1: Summary of morphological and Biochemical characteristics of the three *Leuconostoc mesenteroides* bacteria isolates

Name of isolate	Kakamega						Nyando						Sony					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Elevation of the colony	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
Margin of the colony	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
The surface of the colony	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny
The opacity of the colony	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent
Colour of the colony	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy
Texture of the colony	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy
Gram stain reaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cell shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Catalase activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gas from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fermentation of different sugars																		
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE 1: Contd.

Name of isolate	Kakamega						Nyando						Sony					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Maltose	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Key: + = Positive; - = Negative

Haze method (Chayavanich *et al*, 2024), and pH was measured using a pH meter (inoLab, pH 7310).

Statistical analysis. The data on sugar cane juice quality were subjected to analysis of variance (ANOVA) adapted for a randomised complete block design, using Predictive Analytics Software (PASW). The differences between significant means were separated using the Tukey Honestly Significant Difference procedure at ($P<0.05$) significance.

RESULTS AND DISCUSSION

Identification of *Leuconostoc* bacteria. Examination of sugar cane juice incubated for 24-48 hrs revealed mucous-rich colonies, generally circular, convex with flat edges, smooth, shiny and semi-transparent; all typical of the presence of *Leuconostoc* spp. (Fig. 1). The isolates were catalase-negative when their colonies were exposed to 3% hydrogen peroxide. Further tests revealed that all isolates produced gas trapped in an inverted Durham tube after glucose fermentation, indicating the heterofermentative nature of *Leuconostoc mesenteroides* bacteria (Leisner *et al.*, 2005). For confirmatory identification, the carbohydrate fermentation profile indicated that the bacterium isolated from the 24-48 hr incubation utilises glucose, maltose, sucrose, fructose, galactose, lactose and trehalose as the carbon sources. These characteristics confirmed the identity of the *Leuconostoc* spp. isolates found in sugarcane juice, which agrees with (Bergey, 1994).

Effect of different isolates on selected juice properties. The results showed significant differences ($P<0.05$) among brix% juice, pol% juice, and dextran produced by the three isolates (Kakamega, Nyando, and Sony sugar). These effects were also confirmed by the pH values of the sugar cane juice samples (Table 2). However, the isolate Kakamega and Nyando did not exhibit differences in their performance. The observed differences could

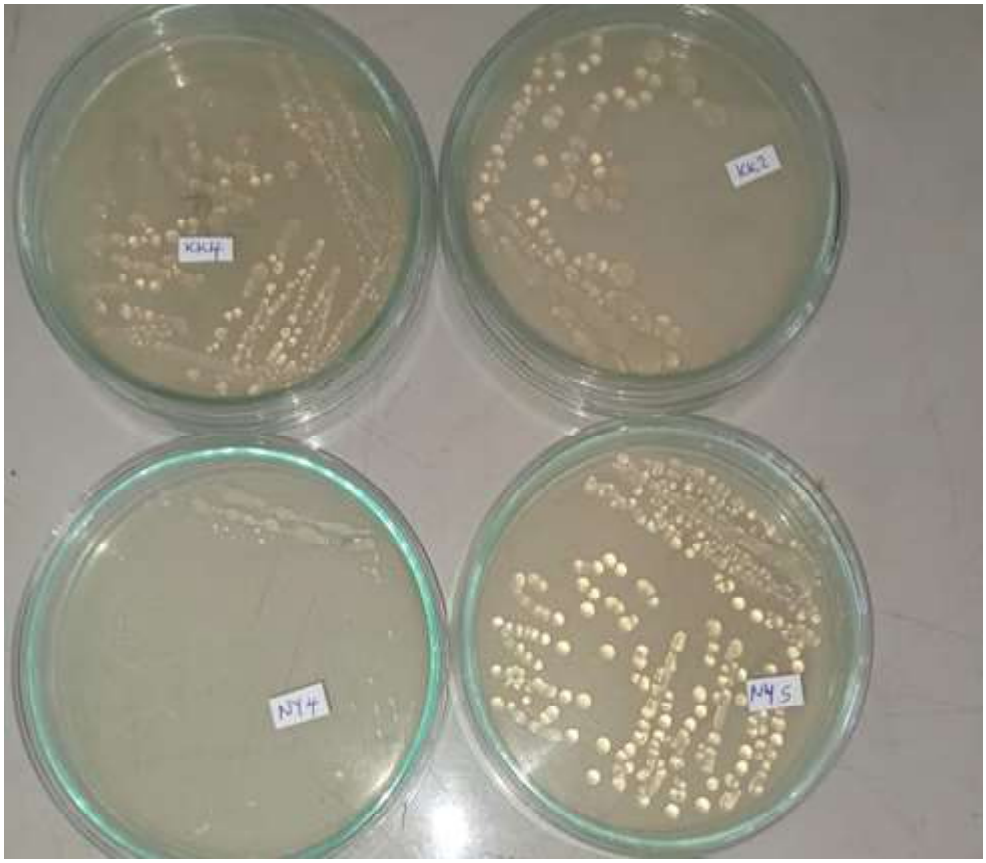

Figure 1. Colonies of the *Leuconostoc* spp. on sucrose and sodium azide agar media

Key: kk2 Kakamega isolate 2, Kk4 Kakamega isolate 4, NY 5 Nyando isolate 5.

TABLE 2. The mean±SD of Brix% juice, Pol% juice, dextran, and pH of sugarcane inoculated with three isolates of *Leuconostoc mesenteroide* from Kakamega, Nyando and Sony

Isolate	Brix % juice Mean±S.D	Pol% Juice Mean±S.D	Dextran (ppm) Mean±S.D	pH Mean±S.D
Control (uninoculated)	20.04±0.70[a]	18.28±0.93[a]	56.94±5.38[c]	6.13±01[a]
Kakamega isolate	19.49±1.19[b]	17.32±1.58[b]	820.95±12.03[b]	5.43±0.61[b]
Nyando isolate	19.35±1.05[b]	17.27±1.55[b]	820.39±12.03[b]	5.38±0.52[b]
Sony isolate	19.29±1.06[b]	17.09±1.61[b]	1654.99±23.02[a]	5.42±0.54[b]
N	72	72	72	72
Df	3	3	3	3
F-value	8.04	9.92	50.2	39.93
P-Value	P<0.001	P<0.001	P<0.001	p<0.001

Means (column) that were marked with different alphabetic letters (a-c) were considered statistically significant differences (P< 0.05)

be associated with the adaptation of the isolates to the different ecological niches of collection (Chase *et al.*, 2021). These variations in microorganisms have been documented to arise from environmental survival pressures, which ultimately introduce heritable traits into a microbe's genome, resulting in differences in resource competition (Nguyen *et al.*, 2021). The observed non-significant differences between Kakamega and Nyando isolates in performance can be attributed to the proximity of the regions to each other, leading to high exchange of sugarcane germplasm between the two areas; resulting in a relative spread of a homogenous population of the bacteria (Saini *et al.*, 2020). Moreover, in recent years, factories in Kakamega have been encroaching on the Nyando sugar belt for sugar cane due to a shortage supply of raw materials (Wanga-Odhiambo, 2016). As a result, this poses another likely route of spread of the isolates in the two regions.

Effect of isolates on brix%. Brix percentage juice represents total dissolved solids, including all sugars and non-sugars in the juice. Generally, a decrease in brix% was observed for all the genotypes under evaluation (Table 3). Juice from Co421 showed the lowest rate of decline in brix% juice over 240 hours of sugar cane storage; while the highest rate of decrease in brix% %juice was witnessed in KEN82-808 (Table 4). The decline in total dissolved solutes indicates that solids were being utilised by the *Leuconostoc* spp. as a biomass source and converted into a liquid and/or gas state. The observations of a decrease in brix% were also reported earlier by Eggleston (2002). The present study results, however, differs from other studies done by Khan *et al*. (2020) and Misra *et al*. (2020); where they reported an increase in brix% juice over time. The difference could be because our experiment was done under a controlled environment, where evaporation was restricted. In contrast, the other experiments were performed in an open environment, where evaporation was very high.

Effect of isolates on pol% juice. The pol% juice, estimates the sucrose content in sugar cane juice. However, using it to quantify the sucrose in deteriorating sugar cane is known to be misleading (Bashari, 2023). Deteriorating sugar cane contains dextran, leading to false polarisation. This is because dextran has a dextrorotatory property that causes it to polarise roughly three times more than sucrose,

TABLE 3. The mean±SD of Brix% juice, Pol% juice, dextran, and pH produced by different sugar cane varieties in Kenya

Variety	Brix % juice Mean±S.D	Pol% Juice Mean±S.D	Dextran (ppm) Mean±S.D	pH Mean±S.D
CO421	20.47±0.59^{a}	18.7±0.83^{a}	306.27±11.36^{b}	5.62±0.54^{a}
KEN 82-808	18.59±0.78^{d}	16.23±1.11^{d}	512.37±15.51^{ab}	5.61±0.54^{a}
KEN83-737	19.76±0.72^{b}	18.11±1.18^{b}	457.82±15.90^{b}	5.61±0.58^{a}
N14	19.33±5.1.05^{c}	16.93±1.47^{c}	871.59±24.48^{a}	5.52±0.63^{a}
N	72	72	72	72
Df	3	3	3 3	
F-value	69.08	65.87	5.91	0.43
P-Value	P<0.001	P<0.001	P=0.001	0.73

Means within a column that are marked with different alphabetic letters (a-d) were considered statistically different (P< 0.05)

TABLE 4. The rate of incline or decline in Brix% juice, Pol% juice, dextran, and pH produced by KEN83-737, KEN82-808, N14, and Co421 varieties

Variety	Brix % juice	Pol% Juice	Dextran	pH
CO421	-0.006	-0.01	7.85	-0.009
KEN82-808	-0.014	-0.017	13.82	-0.009
KEN83-737	-0.01	-0.016	14.55	-0.01
N14	-0.005	-0.017	16.5	-0.011

+ = incline, - = decline

resulting in a high false pol value (Bashari, 2023).

In the present study, a gradual decrement in pol% juice of the evaluated genotypes was observed over the whole staling period (Table 3). Co421 showed an excellent response against sucrose deterioration. The pol% juice values were not only highest for this varieties during 240 hours of storage, but also showed a minimal reduction rate for pol% juice change with a deterioration rate (Table 4). KEN82-808 showed the widest change in pol% juice (Table 3). Although N14 recorded a 1.92 change in pol% juice from 24 hours and 240 hours of storage, it had the same deterioration rate as KEN82-808 (Table 4). KEN83-737 showed a moderate change in the sucrose from 24 to 144 hours of cane storage with a difference of 1.66 and a deterioration rate of (m = -0.016). The decrease in sucrose resulted from the inversion of sucrose to fructose and glucose by the *Leuconostoc* bacteria. The glucose moiety was used for metabolism and to produce dextran by the dextransucrase enzyme; while the fructose moiety was used to produce mannitol (Cogan and Jordan, 1994). It was also noted that some roots sprouted on day 4, using sucrose as an energy source. The results of our study agreed with previous studies by Khan *at al*. (2020), which reported that the cane degradation over storage time led to a loss of sucrose content.

Effect of isolates on juice dextran. A significant increase in dextran was observed across all sugar cane varieties over time (Table 3). The control produced the least concentration of dextran, which began accumulating from 144 hours of storage (Table 2). This could be attributed to contamination of the sugar cane by *Leuconostoc* spp. through handling during the study period. This scenario supports *Leuconostoc* spp. producing dextran in harvested sugar cane as reported by Misra, *et al.* (2020). The highest dextran formation occurred in N14; while KEN 83-737 and KEN 82-808 showed comparatively lower increases in dextran content after 240 hours of storage (Table 3). However, the lowest rate of dextran formation was seen in Co421 after 240 hours (Table 4). Juice from N14 exhibited the greatest rate of dextran increase, followed by KEN83-737, and KEN82-808 (Table 4).

The increase of dextran is attributed to the action of dextransucrase enzyme secreted by the *Leuconostoc mesenteroides* responsible for dextran production (Misra *et al.*, 2020). It is known that for every ppm of dextran produced, there is a loss of 0.0025 pounds of raw sugar (Misra *et al*., 2020). Based on this, it is estimated that N14 lost 2195.38 grammes of sucrose due to conversion into dextran, KEN82-808 lost 1954.98 grammes, KEN83-737 lost 1900.55 grammes pounds, and Co421 lost 1093.16 grammes after 240 hours of storage. These findings align with previous studies by Khan *et al*. (2020), which observed increases in dextran among different varieties over time.

Effect of isolates on juice pH. An increase in acidity of the sugarcane juice was observed over time (Table 3) in all the sugarcane varieties during storage. Nyando isolate produced the lowest pH value, followed by Sony sugar, Kakamega and the control, respectively (Table 2). This is an indication of a reducing pH due to the lactic acid accumulation as a result of *Leuconostoc* spp. metabolising the simple sugars (Wang *et al.*, 2021). In efforts to manage this bacteria, an approach that maintains an almost neutral pH should be sought without compromising the quality of sugarcane juice. N14 recorded the widest margin of change in pH, while the other varieties maintained a similar change trend (Table 3) for the entire storage duration. However, it was clear from regression analysis that N14 had the highest rate of change in pH, followed by KEN83-737 (Table 4). *Leuconostoc* is a lactic acid bacterium (LAB) and prefers an initial medium pH of 6.5 for its growth (Schillinger and Holzapfel, 2011). *Leuconostocs*, like other LABs, do not contain a tricarboxylic acid cycle or a cytochrome system and cannot derive energy from oxidative phosphorylation (Cogan and Jordan, 1994; Folch *et al*., 2021). Instead, they derive energy through substrate-level phosphorylation during the fermentation of sugars into lactic acid, ethanol, acetate, and CO_2 (Folch *et al*., 2021). Production of these acids lowers the pH of the sugar cane juice). These results agreed with the study of several earlier report (Pandraju and Rao, 2020; Panigrahi *et al.*, 2021; Zaidan *et al.*, 2021;), where the pH of sugar cane juice drops with an increase in the number of storage hours.

CONCLUSION

Based on this study, three isolates of *Leuconostoc* spp. were isolated and characterised. The isolates did not exhibit significant differences in their biochemical characteristics. However, in their degradation potentials, some variations were observed where; Sony sugar isolate seemed to superiorly degrade sugar cane juice compared to the other two isolates that had lower but same levels of degradation.

Therefore, strategies to control sucrose loss in harvested sugarcane should be region-specific. N14 and Co421 were the most and least degraded sugarcane varieties. As a result, N14 should be considered for planting in fields near factories to minimise sucrose loss. A molecular characterisation of these isolates is recommended.

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