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Assessment of *Leuconostoc* spp. invasion in standing sugarcane with cracks internode

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Abstract

Aim: The post-harvest deterioration is most vexing problems of sugar industry and these losses due to *Leuconostoc* spp. cause enormous depreciation in cane tonnage and recovery. Thus, the objective of this study was to assess the invasion of *Leuconostoc* spp. in sugarcane.

Methodology: Single internode is divided in three layers, viz., rind, first half and second half. *Leuconostoc* spp. was grown and identified on specific growth media using juice from three portions of both the internodes (with cracks and without cracks) separately. Total *Leuconostoc* load, bacterial characterization, total soluble solids (TSS), reducing sugars (RS) and dextran were also determined.

Results: The results revealed that cracked portions were incurred with *Leuconostoc*, however, rind layer had highest load followed by first half than the second half. More RS, high TSS and dextrans were quantified in cracked internode than without crack internode. Rind layer reported to have high RS, TSS and dextrans than the other portions.

Interpretation: Canes possessing cracks had higher invasion of *Leuconostoc* spp., RS and dextran content than the ones lacking cracks leading to higher loss in sucrose content. Thus, there is a need to control these bacteria in sugarcane, especially in standing canes.

Key words : Dextran, *Leuconostoc*, Reducing sugar, Sucrose, Sugarcane

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Introduction

Lucrative part in sugarcane commonly outstays with growth cracks and frequently occurs in any crop stage which depends not only on varieties but even on growing conditions. Exposure of canes to freezing is one of the common features for appearance of cracks on internodes (Eggleston *et al.*, 2004), apart from excessive intake of water or nutrients by the crop. Sugarcane cracks may be deep in depth or up to the epidermis surface only (Philippe *et al.*, 2000). These cracks allow increased water loss and expose to various biotic stresses (Sandhu *et al.*, 2016). Exposure of cane internal rich regions due to occurrence of cracks is indeed a feast for invasion of microorganisms, especially bacteria like *Leuconostoc* spp., for which such conditions are favorable for growth and proliferation (Solomon, 2009; Misra *et al.*, 2017). The invasion of microorganisms causes several problems which directly or indirectly affects the crop yield or its final product. One of the most vital and evergreen problem in sugarcane is deterioration of sucrose content in pre- and post-harvest conditions. There are many factors behind it but pre- and post- exposure of sugarcane with microorganisms is crucial amongst all (Solomon, 2009; Misra *et al.*, 2016). The former may be due to occurrence of these cracks on cane stalk while latter due to different harvesting methods applied on canes. Invasion of these microorganisms in cane stalks causes deterioration in sucrose content leading to poor quality of juice either in pre- and/or post- harvest condition (Solomon, 2000). It is well known that such canes are known as sour canes. It is obvious that microorganisms after harvest in sour cane proliferate at a higher rate, much higher than the stale canes. Management techniques in sugarcane has already been developed for controlling diseases, insects and pest's incidence, however, invasion of microorganisms can only be minimized, but not controlled. It is known that sucrose content in canes, possessing growth cracks, are poor and also such internodes cannot be used for planting because sett gets easily exposed to fungal and bacterial infection along with frequent incidence of insect and pests (Bakker, 1999). At times sugarcane possessing corky cracks is often misunderstood with growth cracks, however, they differ from the latter as former may be deep and run through the whole length of internode of sugarcane (Philippe *et al.*, 2000).

Leuconostoc spp. is one of the most important soil borne bacteria and is mainly responsible for the sucrose loss occurring in pre- or post- harvested sugarcane, as these enters easily into damaged cane (Misra *et al.*, 2015). Studies have reported that these bacteria are the sole reason behind the microbial deterioration of damaged sugarcane even though invasion of several other kinds of bacteria are also possible (Singh *et al.*, 2015; Hector *et al.*, 2015). Solomon (2000) and Misra *et al.* (2016) reported that in sour cane/stale canes, presence of these bacteria causes the sucrose content to deteriorate leading to formation of organic acids along with typical smell of sourness. This bacterium is responsible for the production of dextrans (Bukhari *et al.*, 2015;

Faramarzi *et al.*, 2013) and mannitol (Eggleston *et al.*, 2004) in harvested canes, implying that a rapid deterioration in sucrose qualities lowers the sugar recovery (Hector *et al.*, 2015). The formation of dextran not only deteriorates the sucrose content in cane stalks but creates several problems during milling in sugar factories such as increase in viscosity (affects the crystallization rate as rate of heat exchange gets lowered), alteration in sugar crystal shape, etc. (Solomon, 2000). In view of the above, the present study aimed to assess the invasion of *Leuconostoc* spp. from growth cracks in standing sugarcane.

Materials and Methods

Plant materials: The whole single canes cracked at internodes were selected for the study and crack internodes were separated from the stalk. Each cracked internode were cut longitudinally into three parts, viz., outer rind layer, layer next to rind layer and remaining portion, for assessment of invasion of the *Leuconostoc* spp. through these cracks (Fig. 1).

Microbial analyses: Juice was extracted from internodes possessing cracks as well as normal internodes. The latter, viz., normal internodes were considered as control. Juice was extracted with the help of a hand crusher so that the juice is free from extra microbial invasion as cleaning and sanitizing the hand crusher is easier. As already mentioned, the cracked internodes were separated so it would be difficult to extract juice in routine crushers. A 100 µl of extracted juice from cracked internode and normal internode were poured and spread in separate sterile plates containing specific media-tryptone (10 g), yeast extract (5 g), sucrose (100 g), agar (20 g) for 1000 ml of media (Mc-Cleskey *et al.*, 1947) for the growth of *Leuconostoc* spp., which were spread with the help of spreader and the plate was incubated at 30°C for 24-48 hrs for good bacterial growth. The media was sterilized at 121°C for 15 min and prior using this media, 0.005% of sodium azide was added to prevent the growth of other bacteria. Total *Leuconostoc* spp. load was calculated manually.

Further, identification and characterization of *Leuconostoc* spp. colonies were performed as per the Bergey's Manual. As a preliminary test, separate isolated colonies from each plate were tested for Gram's staining and catalase test. Further confirmation of bacteria were performed by biochemical tests following Bergey's Manual (Holt, 2000). Colony characterizations were studied by observing the isolated colonies under magnifying glass (10X).

Biochemical analyses: Juice extracted from cracked internodes and normal internodes were collected separately in clean sterile beakers. Associated biochemical parameters (reducing sugars and dextran) with *Leuconostoc* spp. were also studied. Reducing sugars was estimated following the method of Nelson (1944); Dextran was estimated by the method of Haze (Keniry *et al.*, 1969) whereas total soluble solids were determined by hand refractometer.

Fig. 1 : Longitudinal section of (a) internode with crack (b) without crack.

Fig. 2 : *Leuconostoc* load in (a) crack internode and (b) internode without crack in different parts of single internode (1,4-Rind layer; 2,5-First half; 3,6-Second half).

Fig. 3 : *Leuconostoc* spp. Load in CI and WCI in different parts of single internode.

Statistical analyses : The data were analyzed by appropriate statistical analysis (Gomez and Gomez, 1984) using CropStat 7.2 (2009) programme. The second order statistics were computed following Singh and Chaudhury (1985).

Results and Discussion

The bacterial colonies were Gram positive and showed negative catalase test. Colony characteristics showed that the bacterial colonies were smooth, entire, oval in shape, convex, semi-transparent and mucous rich. Furthermore, biochemical analyses showed that the bacteria were able to ferment glucose, mellibiose, maltose, sucrose, fructose, dextrose and lactose, evolving oxygen gas, but lacked in fermenting starch, salicin, D-ribose, raffinose and aesculin. The above biochemical tests confirmed that the bacterial colonies were *Leuconostoc* spp. as per Bergey's manual (Holt et al., 1994).

A high possibility of *Leuconostoc* bacteria invading through cracks and cut ends has been reported (Solomon, 2009). In cracked internode, of all the three portions, the outer rind layer had the highest *Leuconostoc* load (138×10^3 cfu 100 µl⁻¹), followed by the first half (6×10^3 cfu 100 µl⁻¹) and second half (2×10^3 cfu 100 µl⁻¹). There was a difference of 132 units between the rind layer and first half, while 136 units between rind layer and second half. The difference in *Leuconostoc* load clearly indicated that *Leuconostoc* invaded through the growth cracks appearing on the cane stalk, irrespective of the standing or harvested canes (Fig. 2). However, in the normal internodes, few number of *Leuconostoc* spp. were observed in all the portions (Fig. 3). The presence of *Leuconostoc* spp. in normal internode may be due to its invasion from soil when the setts were planted. This may be associated with the fact that the canes having more cracks in their stalk will have more probability of *Leuconostoc* spp. invasion,

Fig. 4 : Reducing sugars in cracked internodes

Fig. 5 : Reducing sugars in normal internodes.

Fig. 6 : Reducing sugars (mg ml^{-1}) in cracked and normal internode in different parts of single internode.

Fig. 7 : Dextran in cracked and normal internode in different parts of single internode.

thus have higher probability of sucrose deterioration after harvest (Misra et al., 2016; 2017). Sharma and Chandra (2017) reported a new *Leuconostoc* spp. for post-harvest losses that mainly invade through cracks or through cut ends. Samina (2012) illustrated that there are multiple sites like growth cracks, globules rich in sucrose content, regions underneath sheath, etc., for invasion of microbes in the sugarcane. Studies have reported that microbial infection in canes is possible due to damage in cane stalks either by growth cracks on internodes or cut regions in the harvested canes (Solomon 2009; Misra et al., 2017; 2016). Invasion of *Leuconostoc* spp. in stale cane juice has been reported by Singh et al. (2015).

In cracked internodes, of all the three portions, the rind layer (outer) had the highest reducing sugars (of 52.69 mg ml^{-1}), followed by the first half (44.64 mg ml^{-1}) and second half (40.80 mg ml^{-1}). This may be due to more microbes, especially *Leuconostoc* spp. on the rind layer as they found favorable conditions for their

growth and proliferation. However, in normal internodes, of all portions, the first and second half had approximately same reducing sugars (29.35 mg ml^{-1}) with a slight increase in reducing sugars in the second half (32.28 mg ml^{-1}). In different cracked internodes, the reducing sugars of same portion were significantly different ($\text{CD}=14.15$) along with significant difference in different portions ($\text{CD}=20.01$) (Fig. 4). There was even a significant difference in normal internodes of same portions ($\text{CD}=0.99$), as well as among the different portions ($\text{CD}=1.40$) (Fig. 5). On comparing the cracked internodes and normal internodes, reducing sugars were highest in cracked internodes by 23.34 units, 15.29 units and 8.52 units in rind layer, first half and second half, respectively (Fig. 6). The statistical analysis showed that the cracked internodes and normal internodes were significantly different ($\text{CD}=4.26$; $\text{CV}=6.144$). This indicated that sucrose is converted more into glucose and fructose in case of cracked internodes. Studies have reported that there is an association between bacteria and sucrose deterioration (Suman

Fig. 8 : Total soluble solids in cracked internodes.

Fig. 10 : Total soluble solids in cracked internode and normal internode in different part of single internode.

et al., 2000) which is being clearly depicted from the results. Singh and Solomon (2003) had revealed that more the invasion sites for microbes, faster will be the loss of sucrose into reducing sugars, dextran and related polysaccharides. This is also evidently illustrated in the results obtained that the internodes possessing cracks had higher reducing sugar than the normal ones.

Microbial invasion may be associated with the amount of dextran present. The formation of dextran takes place due to conversion of sucrose in the presence of dextranase enzyme. This enzyme is associated with *Leuconostoc* spp. (Solomon, 2009; Misra et al., 2016). In cracked internodes, the rind layer (470 mg l⁻¹) had least amount of dextran followed by first half (688 mg l⁻¹) and second half (1078 mg l⁻¹) (Fig. 7). Even though there is higher microbial growth on the rind layer (favorable conditions for their growth and proliferation) but it may be possible that the

Fig. 9 : Total soluble solids in normal internodes.

formation of dextran could be low due to the less amount of sucrose present in the rind layer in comparison to the left-over portion. However, in normal internodes, all portions had almost similar pattern of dextran concentration (301, 359 and 489 mg l⁻¹ in rind layer, first half and second half, respectively) as in cracked internodes but variation among the portions was less compared to cracked internodes. Although the presence of dextran in freshly harvested sugarcane was negligible, however, in this study there was presence of dextran in freshly harvested cracked as well as normal internodes. Furthermore, the dextran concentration in cracked internodes with respect to normal internodes showed that cracked internodes had higher dextran concentration in comparison to internodes free from cracks (56.14, 91.64, 120.44 % in rind, first half and second half, respectively). This variation in dextran concentration of cracked internodes and normal internodes were statistically significant (CD= 121.93; CV=11.88). This indicated that sucrose is converted more into dextran in cracked internodes. Formation of dextran in sugarcane stalks is a matter of concern for sugarcane millers as it is one of the factors responsible for sucrose deterioration (Eggleston et al., 2009; Solomon, 2009; Aquino and Franco, 2009). Several studies have reported that accumulation of dextran due to *Leuconostoc mesenteroides* (Hou et al., 2018; Katina et al., 2009) leads to increased sugarcane deterioration (Solomon, 2009; Eggleston and Monge, 2005; Eggleston et al., 2009; Aquino and Franco, 2009; Eggleston, 2002). It has been previously reported that production of 0.1 % dextran leads to decrease of 0.04 % sucrose (Solomon, 2000).

In different cracked internodes, there was significant difference in total soluble solids within the same portions (CD=3.14) in different internodes as well as among the different portions (CD=4.44) of same internode (Fig. 8). This was also true in case of normal internodes (CD=0.99; 1.40 in within and among different portions, respectively) (Fig. 9). A decreasing pattern of total soluble solids in the three portions in cracked internodes was observed while it was *vice-versa* in normal internodes (Fig. 10). In cracked internodes, the rind layer and second half had almost

same total soluble solids (23.8 and 24, respectively), but the first half possessed highest total soluble solids (28). In normal internodes, there was no significant difference in total soluble solids among the three portions and possessed almost same amount of total soluble solids in the three portions. Higher increase in total soluble solids was observed in cracked internodes in comparison to normal internodes. This variation was found to be statistically significant ($CD=0.99$; $CV=3.03$). Studies have shown rapid increase in total soluble solids after 240 hrs of harvest, irrespective of the type of cane such as damaged, drought or waterlogging affected, etc. (Misra et al., 2016a; Misra et al., 2016b).

Invasion of microbes through cut/damaged parts is a factor for losses in cane weight and sugar recovery. *Leuconostoc* bacterium invades through the rind of cracks into the stalk interior thus revealing higher probability of faster rate of sucrose deterioration after these canes are harvested.

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