THE CAUSE OF SARKARAN IN SUGARCANE

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Abstract

Sarkaran is a polysaccharide that contributes to viscosity in cane processing and so is linked to associated processing problems such as decreased heat transfer and poor crystallisation rates. It is generally found in poor quality, drought or frost-affected cane.

Results from selected factories over the past five years show that it occurs predominantly in the Midlands cane growing area. It has, however, also been observed in other areas of KwaZulu-Natal (KZN) from time to time as well as in neighbouring countries. The levels appear to be worse in drought seasons. Although the supply of low purity cane is inevitable, factory simulated trials indicate that it is possible to remove sarkaran enzymically and so reduce syrup viscosity slightly.

Several fungi were isolated from poor quality, drought or frost-affected cane from the Midlands. One of these fungi, cultivated from several different cane samples, was the stalk pathogen *Phaeocytostroma sacchari*. This was shown to produce sarkaran from sucrose solutions or mixed juice in the laboratory. This suggests that sarkaran is produced as a result of infection of susceptible cane by *P. sacchari*.

Keywords: sugarcane, polysaccharide, sarkaran, pullulan, fungus, *Phaeocytostroma*

Introduction

Sarkaran is a polysaccharide showing some similarities with pullulan and was named by Bruijn (1970, 1973) who characterised it after isolation from whole stalk cane subjected to long post-harvest delay. The unidentified polysaccharide previously described by Nicholson and Lilienthal (1959) was almost certainly the same. Sutherland (1960a, b) linked the presence of this unidentified polysaccharide to high viscosity syrup.

Pullulans are linear polysaccharides elaborated by fungi such as *Aureobasidium*, *Tremella* or *Cyttaria sp*, in which α-maltotriose residues are linked endwise through α-1:6 bonds (Bender *et al*, 1959; Fraser and Jennings, 1971; Leathers, 2002). Bruijn (1973) did not consider the polysaccharide which he isolated from stale cane as a pullulan since enzymic hydrolysis with pullulanase gave different hydrolysis products. Later Blake and Littlemore (1984a) also noted differences in the NMR spectra for pullulan and sarkaran. However, Catley *et al* (1966) have shown that, depending on the strain of organism, pullulan could contain up to 6% maltotetraose by mass and that the fine structure could vary (Catley and Whelan, 1971). Since then many workers have indicated that the proportion of maltotetraose in pullulan, as well as the molecular weight (MW), can vary considerably and are frequently influenced by prevailing environmental and nutritional conditions (Catley, 1971; Catley and Whelan, 1971; Catley, 1972; Taguchi *et al*, 1973; Miura *et al*, 1977; McNeil and Kristiansen, 1990; Leathers, 2003).

Blake and Littlemore (1984a) established that sarkaran did not only occur in stale cane when they reported its presence in standover cane in Queensland. Typical problems attributed to processing standover cane containing sarkaran include very high syrup viscosities, poor crystallisation rates and gumming of heating surfaces. Sarkaran was suspected of being responsible for the extremely high viscosities experienced in the Midlands during 1999 (Morel du Boil, 2000).

Bruijn (1973) was unable to isolate the cause or causative organism for sarkaran formation. He concluded that, "exhaustive experimentation failed to locate a microorganism which could be responsible for the formation of sarkaran in sugarcane during storage. The fact that dry conditions promote the formation of sarkaran is a further indication that the polysaccharide is not produced by a microorganism. It is suggested that sarkaran results from the action of one or more enzymes in the cane, and that these enzymes become active after harvesting because of a disturbance to the normal metabolic equilibria in the cane." Blake and Clarke (1984b) speculated that sarkaran resulted from microbial infection, but were unable to establish the origin of the glucan.

The severity and extent of the occurrence of sarkaran in South Africa is reported in this paper. The isolation and identification of a fungus which produces sarkaran is described.

Methods and materials

(a) *Sarkaran analysis* – the enzyme-HPAEC method developed to characterise sarkaran was used to analyse the various sugar products and experimental extracts (Morel du Boil, 2000).

(b) *Viscosity* – a Brookfield RVDV III rotational viscometer (Anon, 1996) was used to measure evaporator syrup viscosity using a No 2 spindle. Syrup samples were adjusted to 60° or 64°Bx and measurement temperature was either 20° or 25°C. Apparent viscosity was determined at 100 or 150 rpm such that torque was 40 to 45% full scale, *i.e.* an instrument accuracy of ± 4 mPa.s.

(c) *Enzymic removal of sarkaran and other polysaccharides* – pullulanase (Novo Promozyme 400L[1]) was added to mixed juice (MJ) at 190 PUN/kg Bx.[2] The samples were incubated at 55°C, natural pH and sampled at intervals. Sarkaran was completely removed from syrup samples by adding excess pullulanase ($12*10^3$ PUN/kg Bx) to evaporator syrup samples and incubating for 30 minutes at 55°C.

An enzyme cocktail (pullulanase - $12*10^3$ PUN/kg Bx; dextranase (Genencor dextranex L-4000)[3] – $740*10^3$ DXU/kg Bx; α-amylase (Novo Termamyl 120L)[4] – 25 KNU/kg Bx) was added to syrup and reacted at 55°C for 30 minutes (Anon, 1983, 1989, 1996).

(d) *Culture media* – Potato dextrose agar (PDA) plates were used to culture the cane tissue sections. PDA (Merck) (39 g) was suspended in 1 L distilled water and boiled to dissolve.

[1] Mention of a trade name or trademark does not infer a preference for a particular product or brand

[2] 1 PUN = the amount of enzyme that liberates reducing power equivalent to 1 μmole glucose per minute from pullulan under standard conditions at 40°C and pH 5.0

[3] 1 DXU = the activity which will liberate reducing power equivalent to 0.009 μmoles glucose per minute from dextran under standard conditions

[4] 1 KNU = the amount of enzyme that breaks down 5.26 g starch per hour under standard conditions

The medium was autoclaved at 121°C for 15 minutes, the pH adjusted to 5.6 and plates prepared.

(e) *Sucrose (or mixed juice) broth* - was prepared by adding 1 ml 10% di-ammonium phosphate to 100 ml of 15% sucrose or MJ. The solution was autoclaved at 121°C for 15 minutes. In later trials, the sucrose broth was prepared using 5% sucrose in a 'mimimal salts' medium (2 g K_2HPO_4, 1 g $(NH_4)_2HPO_4$, 0.5 g NaCl, 0.05 g $MgSO_4.7H_2O$, 0.01 g each of $FeSO_4$, $MnSO_4$ and $ZnSO_4$ made to 1 L with distilled water). The pH was adjusted to between 6 and 7 and the medium was autoclaved at 121°C for 15 minutes. Cultures were incubated at room temperature with frequent manual shaking over a period of up to 10 days. Samples were withdrawn daily and analysed for sarkaran and sugars.

(f) *Isolation of organism* – cane tissue was obtained using the procedure described by Goodall *et al* (1999), a general technique for distinguishing pathogens from surface contamination. Pieces of internal internodal tissue (approximately 1 cm^3) were surface sterilized by soaking in 10% 'Jik' (5 minutes), the outer surfaces aseptically removed and the sterilization repeated (2 minutes). The cane pieces were air dried, dipped in 70% ethanol (2 minutes), flamed and plated onto PDA. Plates were kept at 20° to 25°C under natural light. Mycelia growing from the tissue sections were transferred onto fresh potato dextrose agar (PDA) plates and the cultures were maintained by sub-culturing onto fresh PDA plates every second week.

(g) *Industrial samples* - mixed juice samples were the Cane Testing Services (CTS) weekly composites, sugar samples were obtained from South African Sugar Terminals (SAST) or from the individual factories, molasses samples were the SMRI weekly or monthly composites.

(h) *Cane samples* were either (i) healthy or drought stressed cane selected and harvested by the local extension officer or agronomist. A summary of this cane is included in Appendix 1 (Table 1.1). Direct analysis of cane (DAC) extracts were prepared at the SMRI (Anon, 1985), or (ii) stalks from individual consignments selected as the cane was delivered to the factory. A brief description of these samples is included in Appendix 1 (Table 1.2). Since only small amounts of cane were available, the stalks were cut and shredded manually. Warm water (200 ml) was added to shredded cane (100 g). The slurry was stirred for 10 minutes and the juice was extracted by squeezing.

Results and discussion

Occurrence

Sarkaran has been reported throughout the southern African cane growing area and frequently occurs at levels approaching 1000 mg/kg Bx in MJ (Morel du Boil, 2005b). It is particularly prevalent in the KZN Midlands region with concentrations regularly approaching 2000 mg/kg Bx in MJ. This usually occurs when poor quality cane is being processed following severe drought growing conditions and is generally associated with very high processing viscosities.

A typical seasonal trend in sarkaran levels for a bad year is shown in Figure 1. Sarkaran levels ranging from 3 000 to 7 000 mg/kg Bx were obtained on five samples of DAC extract from Zimbabwe during a very severe drought. The corresponding dextran concentrations were less than 300 mg/kg Bx. The historical six-year trend for a Midlands factory is shown in

Figure 2, with the corresponding rainfall pattern given in Figure 3. For much of this period rainfall was below the long term mean (LTM) (Anon (2005), Rainfall data. *http://sasex.sasa.org.za/cgi/~455.mon*).

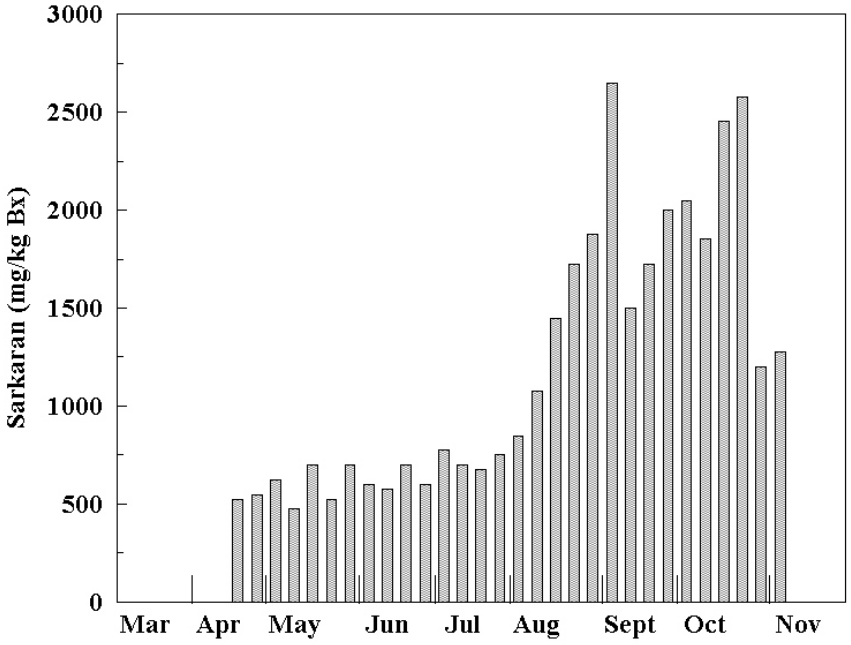

Figure 1. Example of increased MJ sarkaran at end of winter after dry conditions (Midlands factory, 2004).

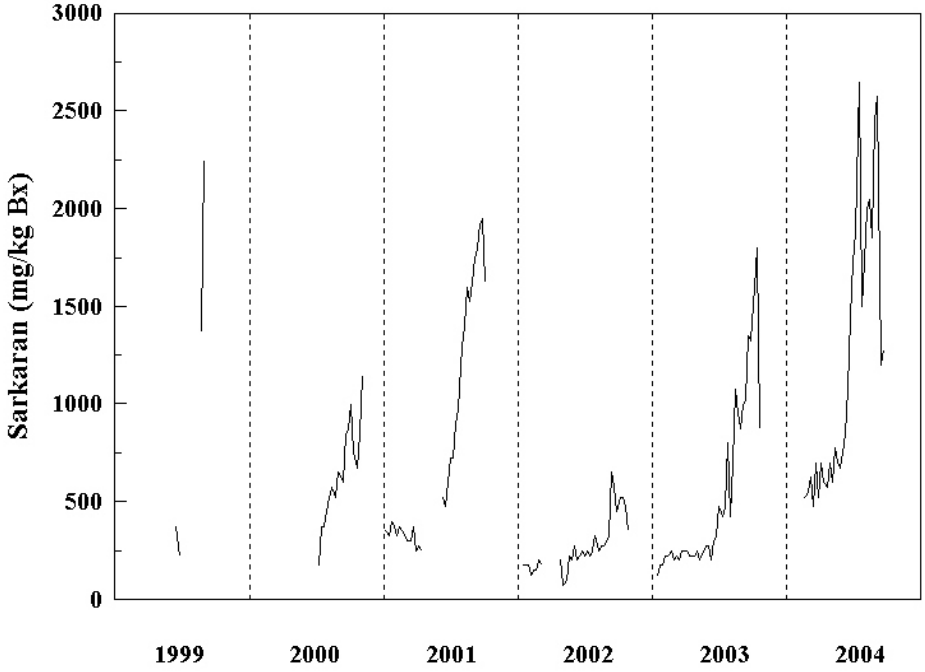

Figure 2. Seasonal trend in MJ sarkaran levels for a Midlands factory (data plotted monthly for each year).

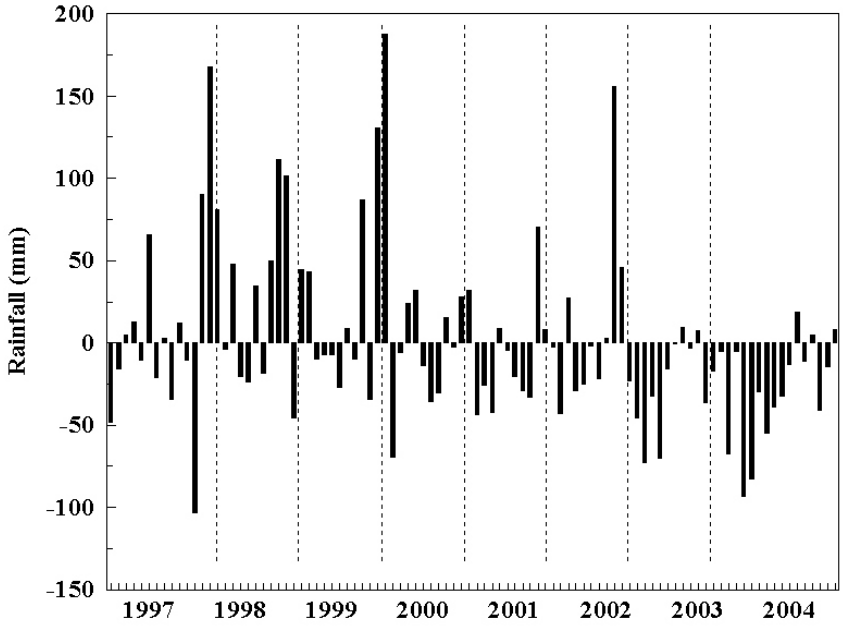

Figure 3. Rainfall – difference from long-term mean for each month for Wartburg weather station.

Sarkaran has been detected in both raw and refined sugar (Morel du Boil, 2000), as well as in molasses (Blake and Littlemore, 1984b; Morel du Boil, 2005b). Although Nicholson and Lilienthal (1959) and Bruijn (1966a) inferred that sarkaran was formed as a result of cane staling, Blake and Littlemore (1984a, b) and Blake and Clarke (1984b) found it to be more widespread. The accumulation of small amounts of sarkaran (about 250 mg/kg Bx) in burnt, cut, whole stalk cane, after about two weeks of storage, has also been reported by Barker (2000).

Sarkaran concentrations tend to peak between October and November (Figures 1 and 2). When a specific analysis for sarkaran became available (Morel du Boil, 2000), final molasses samples from mid-October for some factories were analysed retrospectively for sarkaran. Data are shown in Table 1. Clearly the occurrence of sarkaran (particularly in the Midlands cane growing area) has been of long standing.

The molar ratio of maltotriose to maltotetraose was found to be 1.8 ± 0.3 for 740 MJ samples containing more than 200 mg sarkaran/kg Bx, with 90% of the samples falling in the range 1.6 to 2.5 (Morel du Boil, 2005b). This is similar to the ratios reported by Bruijn (1966b) and Blake and Clarke (1984a, b) and is in agreement with the latter observation that sarkaran produced under different conditions can have significant variation in the maltodextrin building blocks. Sarkaran isolated from cane stored at temperatures above 30°C was found to have about 30 to 40% maltotriose, compared to the 50 to 60% from cane stored under ambient conditions (Blake and Clarke, 1984b).

Table 1. Historical sarkaran levels in final molasses for mid-October (mg/kg sample).

Year	Mill							
	NB	UC	ML	PG	UF	AK	MS	SZ
1991	3575	2275						
1992			1450	775		3950		
1993								
1994	3750	2825	950	575				
1995	4175	2525		775				
1996	2150	3375		650				
1997	3550		425			4450	975	
1998	6650	4650	875					
1999				975				
2000	2300				4550			
2001	6250	4050				5000[a]	5750[a]	
2002		2650						1750
2003						1375	1050	
2004	7850		600					

[a]Sampled in May 2001, *i.e.* carryover cane from previous growing season

Effects on processing

Crystal transfer – analysis of a limited set of mixed juice and VHP sugar (37 sample pairs) indicated that about 9% of the total sarkaran present in mixed juice was transferred to the crystal. Almost half (40%) of the sarkaran in VHP was high MW (Morel du Boil, 2005b). This is similar to the value obtained earlier (Morel du Boil, 2000), but is considerably lower than the corresponding transfer (about 20 to 25%) obtained for dextran (Morel du Boil, 2005a) and probably reflects the higher solubility and lower molecular weight of sarkaran when compared to dextran. Transfer of sarkaran from raw sugar to refined sugar was found to be about 30% based on six monthly composite samples from a carbonatation refinery.

Viscosity – sarkaran can be reduced using an enzyme called pullulanase which is readily available commercially (*e.g.* Novo Promozyme). Sarkaran in MJ was reduced rapidly in laboratory trials at 55°C, natural pH, using 190 PUN/kg Bx (Figure 4).

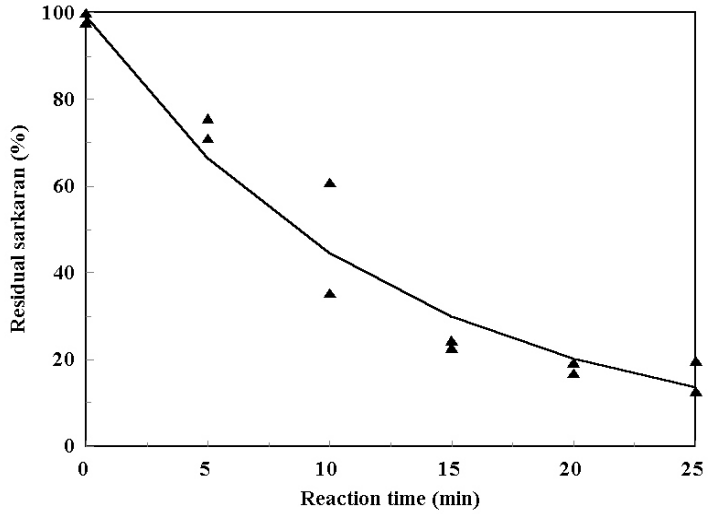

Figure 4. Rate of removal of sarkaran from MJ at 55°C, natural pH using pullulanase at 190 PUN/kg Bx.

Larger doses ($12*10^3$ PUN/kg Bx) and longer reaction times (30 minutes) were used to ensure complete removal of sarkaran so that the effect of sarkaran on viscosity could be estimated. The apparent viscosity of evaporator syrup was measured before and after removal of sarkaran and compared with the calculated viscosity for pure sucrose (Anon, 1994) under the same conditions (Figure 5). Removal of dextran, sarkaran, amylose and some amylopectin from evaporator syrup using an enzyme cocktail in the laboratory gave only a small further reduction of viscosity. This treatment only removed about 40% of the gums (5 100 ppm before treatment and 3 000 ppm after treatment).

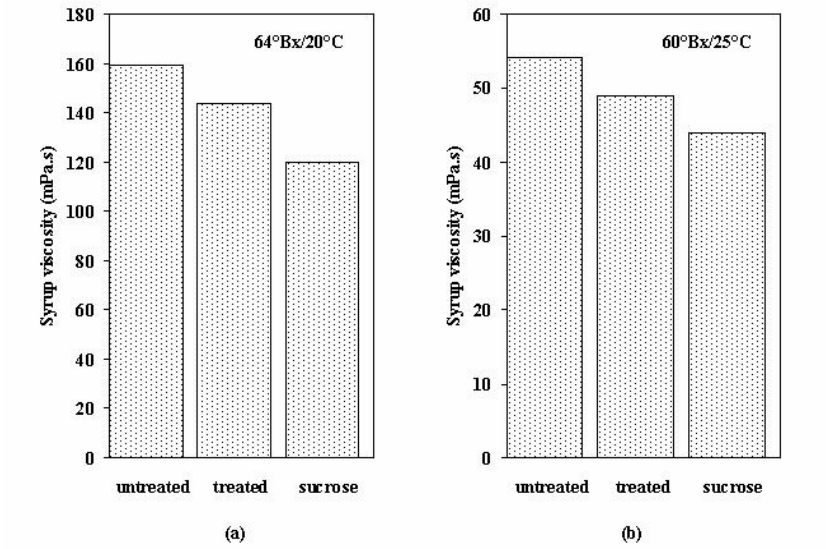

Figure 5. Evaporator syrup viscosity before and after removal of sarkaran at (a) 64°Bx/20°C and (b) 60°Bx/25°C.

Increases in viscosity lead to reductions in throughput and exhaustion. Bruijn (1973) used a viscometric technique (falling ball Hoeppler viscometer) to estimate the MW of sarkaran. The viscosity data indicate that sarkaran solutions are considerably less viscous than the equivalent dextran solutions (limiting viscosity = [η] = 18.4 for sarkaran and 77.6 for dextran). However, the sarkaran characterised by Bruijn (1973) was of relatively low MW (*ca* 50 000 Da). Indications are that sarkaran MW can be considerably higher (*ca* 18 000 to 200 000) (Blake and Clarke, 1984b). Data recorded in South Africa over the past five years using different ethanol concentrations for polysaccharide isolation are consistent with a proportion of the sarkaran being of relatively high MW (Morel du Boil, 2005b). Hence, although Bruijn found the viscosity of sarkaran to be relatively low, higher MW fractions can be expected to give higher viscosities since viscosity is a function of both concentration and MW. Pullulan solutions are generally considered to be of low viscosity (Leathers, 2003), although Wallenfels *et al* (1965) gave the limiting viscosity ([η]) of a pullulan of MW 235 000 as 113.

Although the viscosity data were obtained under less than favourable conditions, it appears that sarkaran *per se* does not cause viscosity increases as high as, for example, similar dextran concentrations (Geronimos and Greenfield, 1978; Greenfield and Geronimos, 1978). Clearly, although sarkaran has been associated with extremely high viscosities, there are other factors contributing to the high viscosity.

Polarisation – although this aspect was not investigated in this study it has been reported that dry lead acetate clarification does not remove sarkaran from juice (Bruijn, 1973; Blake and Clarke, 1984b). The specific rotation of sarkaran has been reported as $[160°]_D^{20}$ (Bruijn, 1973) or $[167°]_D^{20}$ (Blake and Littlemore, 1984a) and so the presence of sarkaran will probably inflate pol readings slightly.

Trials on freshly harvested cane

Preliminary trials carried out in 2000 indicated that drought stressed cane could contain high levels of sarkaran, particularly in the bottom half of the stalk (Appendix 1) (Anon, 2001; Morel du Boil, 2001). Subsequent trials during 2004 confirmed that sarkaran levels were higher in stressed rather than healthy cane (Table 2). In view of this outcome and the fact that relatively high levels of sarkaran were being measured in MJ during September, 2004 (Figure 1), further samples of cane were collected at the factory gate during October, 2004.

Table 2. Analysis of field cane collected September-October, 2004.

Parameter	Trial 3 (September, 2004)		Trial 4 (October, 2004)	
	Healthy	Poor quality	Healthy	Poor quality
	Cane E	Cane F	Cane G	Cane H
Variety	N31	N16	N12	N12
Purity	78.1	54.7	92.7	91.3
Sarkaran (mg/kg Bx)	150	3350	375	2275

For description of samples see Appendix 1

Trials using cane delivered to factory

The sarkaran results on the laboratory extracts for the eight cane samples collected at the factory gate are given in Table 3. Generally, dextran was low or absent.

Table 3. Sarkaran concentrations in laboratory extracts from factory gate samples.

Sample	I	J	K	L	M	N	O	P
Sarkaran (mg/kg Bx)	2475	75	200	100	100	4000	150	100

For description of samples see Appendix 1

Isolation and selection of cultures

Preliminary trials

Cane sample F (see Appendix 1) was used to establish an experimental protocol for selecting sarkaran producing fungi. Cubes of sterilized cane were grown on PDA. Three fungal growths (red, green, grey) were obtained. The mycelia from a single plate of one-week old culture were transferred to 15% sucrose or MJ broth. The transfer was not quantitative since only a qualitative indication of sarkaran production was required. The flasks were shaken frequently and analysed for sarkaran after six days. Only one of the fungi produced sarkaran (Table 4). In all cases dextran was less than 100 mg/kg Bx.

Table 4. Sarkaran production in sucrose or MJ broth for culture from cane sample F.

Parameter	Un-inoculated broth		Inoculated broth	
	15% sucrose	MJ	15% sucrose	MJ
Bx	15.8		16.2	
Sarkaran (mg/kg Bx)	0	650	650	1250

There was little difference in sarkaran production whether an 'old' (black) or 'young' (white) colony was cultured in 5% sucrose-minimal salts broth for five days (450 and 325 mg/kg Bx, respectively).

The rate of formation of sarkaran was monitored after inoculating a 5% sucrose-minimal salts broth with the mycelia from a single plate of week old culture from cane sample F. The conditions were neither optimized nor quantitative. The results are shown in Figure 6a. Sarkaran production reached a maximum after seven days and then decreased. Similar decreases in polysaccharide yields have been reported for pullulan production from *Aureobasidium sp* and have been attributed to the production of extracellular enzymes (Campbell *et al*, 2003). Sucrose was completely inverted within seven days and the total sugars decreased by 15%. Further confirmatory trials on the sarkaran producing potential of the fungus were conducted for seven days.

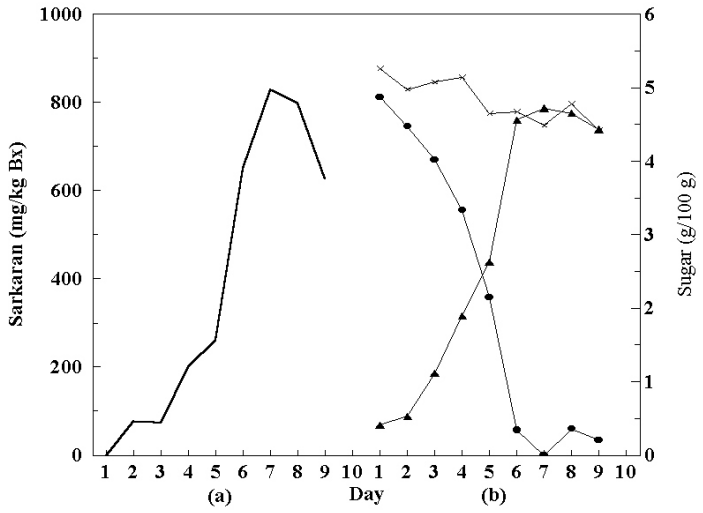

Figure 6. Accumulation of sarkaran (a) and inversion of sucrose (b) during growth of fungus in 5% sucrose-minimal salts broth (● sucrose, > invert sugar, x total sugars).

Confirmation of sarkaran production

Several sections from each of the eight samples collected at the factory gate as well as from the four samples of field cane harvested in September and October, 2004 were cultured on PDA (a medium selective for yeasts and moulds). Fungal growth was observed on 23 of the 59 isolates. These were transferred into 5% sucrose broth-minimal salts broth and analysed for sarkaran after seven days incubation. Sarkaran was produced by 13 of the 23 isolates after seven days incubation at ambient temperature (Table 5). This represented six different cane samples (varieties N12 and N16).

Table 5. Sarkaran production in 5% sucrose broth with minimal salts after seven days' incubation under ambient conditions.

Cane sample	Isolated from	Sarkaran (mg/kg Bx)
F		500
H	Short internodes	225
	Very dry; sour smell	400
	Reddish	550
	Reddish	675
	Streaked grey fungus	650
	Reddish internode	300
	Ring of cane	325
I	Short internode; freeze regrowth	925
	Black red streak	250
L	?	225
M	Just above freeze 'branch'	125
N	Below freeze regrowth; brownish	1675

Invert formation

As the onset of sarkaran formation coincided with the formation of invert sugars (in either 5% or 15% sucrose solution) (Figure 6b), an autoclaved mixture of glucose and fructose (2.5% (w/v) of each) with minimal salts was inoculated and the broth was sampled daily. No evidence of sarkaran formation was recorded after 10 days. That sucrose appears necessary for sarkaran formation contrasts with reports that pullulan can be produced from several substrates (Leathers, 2002). It is possible that fermentation toxins were produced during the sterilization of monosaccharide solutions and this preliminary indication that the culture does not produce sarkaran from glucose or fructose needs to be confirmed.

The production of invert sugars indicates that the organism probably secretes invertase. This will have a major impact on cane purity in infected cane.

Description of fungus

Plate culture on PDA – A white mycelium (diameter 10 mm) was visible within two days, increasing to about 40 mm by day four. At this stage the colour of the colonies began darkening from white to grey and ultimately dark grey. On day five clear droplets were visible under the stereomicroscope. As the colonies aged, the colour of the exudates changed from clear to gold to black and the size increased, becoming visible to the naked eye within about 10 days. These turgid, fragile, black spore masses released ellipsoid spores (10-12 μm in length) after three to four weeks (Figure 7).

Flask culture – When grown in sucrose or mixed juice broth the solution showed thickening with the formation of jelly-like masses adhering to the glassware. Such behaviour is frequently observed with extracellular polysaccharide formation and is associated with mycelial mats (Leathers, 2002).

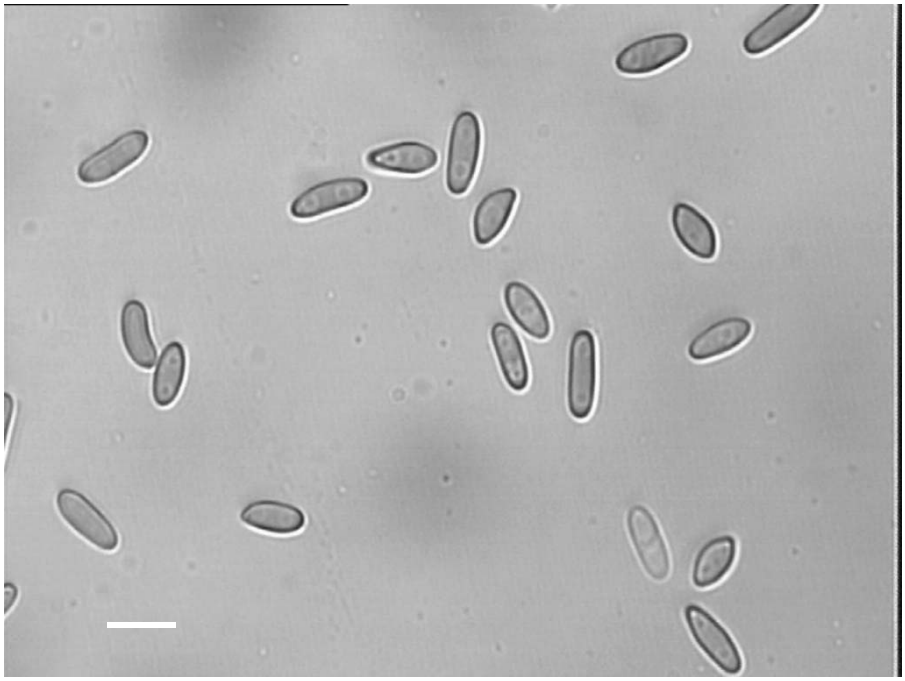

Figure 7. Fungal spores (bar = 10 μm).

Cane – Cane stalks inoculated at the South African Sugarcane Research Institute (SASRI) produced the black spore masses diagnostic for *Phaeocytostroma sacchari*[5].

Identification of fungus

Based on the morphology of the culture on PDA, the spores and the cane trials, the fungal culture was identified as *Phaeocytostroma sacchari* Sutton by SASRI[5]. This pathogen and the stalk rotting disease that it causes have most recently been described by Comstock and Bailey (2000). The fungus was also positively identified by the Centre for Applied Mycological Studies (CAMS) as *P. sacchari* - "the fungus produced typical eustromatic conidiomata and the dark coloured ameroconidia are imbedded in the exudates. The isolates were compared with *P. sacchari var. penniseti* and *P. sacchari var. calamari*, but the size of the spores is typical of *P. sacchari*."

The fungal pathogen *P. sacchari* has previously been reported from the Midlands cane growing area and is responsible for rind disease and sour rot. Regional surveys conducted by SASRI showed that the disease was particularly prevalent after prolonged drought conditions and led to low purity juice with substantial losses in sucrose yield (Goodall *et al*, 1999; McFarlane and Bailey, 2001). The physiology of *Pleocyta sacchari* (a synonym for *Phaeocytostroma sacchari*) has been discussed by Liu *et al* (1977). The fungus is found globally (Comstock and Bailey, 2000).

It is believed that this is the first published observation linking *P. sacchari* to the production of the polysaccharide sarkaran.

[5] Personal communication, Sharon McFarlane, Pathologist, SASRI

Conclusions

The widespread occurrence of sarkaran within the industry has been established. Although it occurs mainly in the Midlands, it has also been recorded in samples from neighbouring countries. It is generally found in poor quality cane of low purity and is associated with extremely viscous process streams. However, it appears that other factors also contribute to the relatively high viscosities encountered since removal of dextran, sarkaran, amylose and some amylopectin, using an enzyme cocktail in the laboratory, only gave a small improvement in viscosity whilst only accounting for 40% of the gums in evaporator syrup. Laboratory trials indicated that pullulanase could be used to remove sarkaran from mixed juice, but the economics have not been considered. The benefit to be gained by reducing viscosity does not appear to be worthwhile. Sarkaran is particularly prevalent in drought seasons and has been observed throughout the factory, as well as in refined sugar. The MW and the ratio of the component maltotriose and maltotetraose sub-units show some variation although the distribution of this ratio is remarkably narrow. In the main, sarkaran is a function of the cane quality at harvest, since it is prevalent in drought and disease-stressed cane. Sarkaran also continues to be formed post-harvest under dry conditions.

The major source of sarkaran is most probably a result of fungal infection in the field. The causal agent has been isolated from sugarcane and has been identified as the fungal pathogen *Phaeocytostroma sacchari*. It has been shown to produce sarkaran in sucrose or mixed juice solutions in the laboratory. The accumulation of sarkaran is paralleled by the reduction of sucrose and an increase in reducing sugars. This will have a major impact on juice purity. *P. sacchari* is known to be widespread, both locally and globally, when conditions (weather and variety) are suitable and it may not occur in isolation. It is possible that other fungi and bacteria produce polysaccharides that contribute to viscosity and hence might have an impact on cane quality, factory processing and sugar quality. As far as is known, *P. sacchari* has not previously been implicated in the formation of sarkaran.

Acknowledgements

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APPENDIX 1

Table 1.1. Summary of sampled field cane.

Trial	Sample	Harvested	Variety	Purity	Comments
1	A	12/09/00	N12	92.6	growing well
	B			63.0	drought stressed; signs of stalk rot
2	C	21/09/00	N12	91.5	growing well
	D			67.8	normal die-back rather than stalk rot
3	E	31/08/04	N31	78.1	annual cane, burnt and cut 31/8
	F		N16	54.7	burnt 30/8, cut 1/9 – some stalk rot
4	G	12/10/04	N12	92.7	cane visually in excellent condition
	H		N12	91.3	stunted growth, very dry, earthy, sour smell

Table 1.2. Summary of cane delivered to the factory.

Sample	Burnt	Cut	Variety	Purity[a]	Comments
I	8/10/04	9/10/04	N16	88.8	some freeze damage
J	8/10/04	9/10/04	N16	90.2	few splits
K	11/10/04	11/10/04	N12	87.9	many lengthwise splits; brown-orange
L	11/10/04	12/10/04	N16	88.1	normal looking
M	9/10/04	11/10/04	N12	87.3	some freeze damage, dessicated
N	11/10/04	12/10/04	N12	83.1	freeze damage, corky nodes
O	9/10/04	12/10/04	N16	86.0	slight damage
P	11/10/04	12/10/04	N29	-	normal looking

[a] analysis of consignment, not of selected stalks

Table 1.3. Example of sarkaran distribution in healthy versus drought stressed cane (N12, harvested September, 2000).

Parameter		Trial 1 (12/9)		Trial 2 (21/9)	
		Healthy	Drought stressed	Healthy	Drought stressed
Average stalk length (m)		1.77	1.13	1.74	1.10
Average diameter (cm)		23	20	27	20.5
Cane mass (kg) (number stalks)		21.3 (29)	11.9 (27)	18.6 (24)	12.5 (22)
Purity		92.6	63.0	91.5	67.8
Sarkaran (mg/kg Bx) – whole stalk		100	2850	0	260
	top	200	175	0	270
	middle	50	175	-	-
	bottom	75	6325	0	250
DAC:	Bx	6.2	5.8	6.2	5.4
	Fructose (g/100 g Bx)	<0.1	0.4		
	Glucose (g/100 g Bx)	<0.1	0.4		
	Sucrose (g/100 g Bx)	6.2	3.6		