REFEREED PAPER

MICROBIAL DIVERSITY PROFILING IN SUGARCANE PROCESSING: WHAT, WHY AND HOW?

NEL S

Sugar Milling Research Institute NPC, c/o University of KwaZulu-Natal, Durban, 4041, South Africa
snel@smri.org

Abstract

Sugarcane and some locations in a sugarcane processing factory provide an attractive environment for the growth and reproduction of microorganisms, in particular gum-producing bacteria. The presence of microorganisms in a sugar factory is undesirable due to their actions; not only do they result in a direct loss of sugar, they can also lead to higher production costs due to the detrimental effects of gums produced by microorganisms in the sugar manufacturing process. It is vital to establish the identities and locations of gum-producing microbes in sugarcane processing to be able to develop a targeted approach to eliminate these organisms and their actions from the sugar manufacturing process. The aim of this paper is to enlighten the reader on the topic of microbial diversity profiling, with specific application to the sugarcane processing industry; in particular, explaining *what* this term means, *why* it is of importance and *how* this could be executed.

Keywords: gums, post-harvest cane deterioration, *Leuconostoc*, dextran

Introduction

The physical and chemical properties of sugarcane juice make it an excellent substrate for the proliferation of a variety of microorganisms which ultimately cause sucrose degradation and the production of microbial metabolites such as acids (lactic and acetic acid), alcohols (mannitol and ethanol) and polysaccharides (levan and dextran) in the sugarcane juice (Cerutti de Guglielmone *et al.*, 2000). The time lag from burning/cutting to crushing (burn/harvest to crush delay or BHTCD), together with suitable environmental conditions, provide ample opportunity for the proliferation of microorganisms (Ravnö and Purchase, 2005; Solomon *et al.*, 2008). It is widely reported that the presence of high concentrations of gums in sugarcane and subsequent processing streams adversely affects sugarcane processing and yields and quality of the produced sugar (Cuddihy Jr *et al.*, 2001; Ravnö and Purchase, 2005). Cuddihy Jr *et al.* (2001) defined 'gums' as "*carbohydrates of high molecular weight which are precipitated from aqueous solutions by acidified ethanol*." In sugarcane factory streams and products, these gums may include carbohydrates from the sugarcane plant's structure (hemicellulose, pectin) and metabolism (starch), and in the case of deteriorated cane, polysaccharides (dextran, levan, sarkaran) produced by microorganisms.

In 1972, Imrie and Tilbury stated that the harmful effects of polysaccharides in sugarcane processing only receive attention after abnormally high gum contents are experienced, often when sour or stale cane is milled. In the same paper the authors commented that, "*Further study of the harmful effects of polysaccharides in processing and methods of minimising these appear to be a worthwhile topic of research*." In 2001, Cuddihy Jr and co-workers remarked that, although the above quote was documented in 1972, the sugar industry was still

struggling with these problems. A paper in 2005 by Ravnö and Purchase reinforced these statements by reporting on the growing realisation that dextran has become a more serious sugar quality issue in South Africa and that "*The first priority should be to adopt practices that prevent or minimise the incidence of high dextran levels entering the boiling house*." However, according to Solomon *et al.* (2008), "*Despite huge monitory* (sic) *losses to the sugar industry, the management approach to curb post-harvest sucrose losses is almost non-existent*." The topic of gums in sugarcane processing streams and its effects continued to receive attention with a recent study carried out at the Sugar Milling Research Institute (SMRI) which involved the quantification and analyses of the gums isolated from final molasses (du Clou and Walford, 2012), demonstrating that the 1972 quote by Imrie and Tilbury is still valid today. So why has this decades-old problem of gums in sugarcane processing not yet been solved? Kulkarni (1999) may have recognised one part of the answer to this question when he remarked, "*There is tremendous lack of understanding about mill sanitation in general and biocides used in particular in the sugar industry. The confusion is because of lack of adequate knowledge about microbes and enzymes present in cane and juice, which are responsible for major losses in recoverable sugar*." An additional factor is the lack of knowledge and understanding of the microbial diversity of the organisms responsible for gum production in the various processing streams.

This paper advocates the concept of microbial diversity profiling of gum-producing microorganisms in sugarcane processing. Profiling may be used as a tool to ultimately enable the development of a targeted approach to eliminate particular organisms (which are confirmed gum-producers) from specific locations in sugar factories and processing streams to subsequently reduce sucrose losses and the associated adverse effects on sugarcane processing.

Microbial diversity profiling of gum-producing microorganisms in sugarcane processing – *what* does this mean?

The word 'profiling' conjures up images of TV programmes and movies on serial killers, the FBI and so forth. Muller (2000) defined criminal profiling as, "*The process of using available information about a crime and crime scene to compose a psychological portrait of the unknown perpetrator of the crime.*" It is tempting to use this definition as an analogy for microbial diversity profiling; "The process of using the available information (*and the accumulation of novel data*) about the crime (*viz.* the loss of sucrose by the action of microbes, which then use it to produce gums, as well as the adverse effects of these gums on processing) and crime scene (*viz.* the various sugarcane processing streams) to compose a *scientific* portrait of the (*as yet*) unknown (*but will be identified*) perpetrator of the crime (*viz.* gum-producing microorganisms). "Simply put, microbial diversity profiling will reveal the identities of the microbes responsible for gum production from sucrose at specific locations in the sugar manufacturing process. Furthermore, these gums can be isolated from their producing microorganisms, and the gums can then be quantified and evaluated in terms of their physical and chemical characteristics.

Microbial diversity profiling of gum-producing microorganisms in sugarcane processing – *why*?

The history of the identification of gum-producing microorganisms in sugarcane processing dates back to the 1800s, when Louis Pasteur first reported on slime production by small cocci in sugar-containing liquid (Pasteur, 1861). The name 'dextran' was introduced by Scheibler

in 1874, when he discovered that the mysterious thickening of cane and beet sugar juices was caused by a carbohydrate of empirical formula ($C_6H_{10}O_6$) with a positive optical rotation (Scheibler, 1874). Shortly thereafter it was suggested that *Leuconostoc* was responsible for slime production in sugar factories (Van Tieghem, 1878). Today we know that dextran (and other gums) can be produced from sucrose by several bacterial species and that the structure of each type of gum depends on the microbial strain that produces it (Kralj *et al.*, 2004; Bounaix *et al.*, 2010; Lakshmi Bhavani and Nisha, 2010; Palomba *et al.*, 2012; Paulo *et al.*, 2012; Vettori *et al.*, 2012).

To answer the question "Why do we need to profile gum-producing microorganisms from sugarcane and its processing streams?" it may be prudent to establish some rudimentary understanding of the pertinent dynamics involved and to question historical assumptions.

Where do these gum-producing microorganisms come from?

The origin of the contaminating microorganisms has been traced to the soil adhering to the sugarcane stalks and leaves. Upon harvesting, the cut ends of the cane stalks are open to microbial infection originating not only from the soil and surrounding plant matter, but also from the contact with contaminated cane knives. Cutting of cane is not the only mode of infection; whenever a stalk is damaged either by borer, burning or frost, it is exposed to microbial life. If there is any rain-induced BHTCD in combination with suitable environmental conditions, microorganisms will proliferate. Ravnö and Purchase (2005) commented on the close correlation between rainfall and increased levels of dextran; these authors suggested that this correlation is mainly an indirect result of BHTCD. It has been shown that burnt, wet cane deteriorates more rapidly than burnt, dry cane (Atkins and McCowage, 1984; Anon, 1993). This is in agreement with the hypothesis that burnt cane is often partially covered externally with juice, which then supports extensive microbial growth if the cane is not allowed to dry out immediately after burning (Ravnö and Purchase, 2005). During sugarcane processing most of the microorganisms which infect the cane stalks are washed off into the extracted juice; this is an excellent substrate for microorganisms in which they utilise the sucrose for growth with the concomitant excretion of their metabolic products (acids, alcohols and polysaccharides) into the surrounding environment.

It is worthwhile to remember that inadequate housekeeping and hygiene inside a factory and refinery might also result in the contamination of processing streams with microorganisms which can result in sucrose losses, gum production and related processing problems.

What is the significance of microbial infection and contamination on sugar production?

The mere presence of microorganisms in/on sugarcane and in the sugarcane factory is not the main issue; it is what these organisms *do* in/on the cane and process streams that has caused many headaches for many years in sugarcane processing industries worldwide, and still continues to do so today.

As mentioned earlier, contaminating microorganisms use sucrose to sustain their metabolic processes, and in return produce gums, alcohols and/or organic acids; these products of microbial metabolism are then excreted into the surrounding environment (i.e. the sugarcane stalks and relevant processing streams). Two major actions of contaminating microorganisms are (i) the removal of sucrose from the sugar production process (by using it to grow and reproduce), directly resulting in reduced sucrose availability for sugar production, and (ii)

producing gums from the sucrose, resulting in a multitude of problems in sugarcane processing. Imrie and Tilbury (1972) described extensively the adverse effects of gums on sugar manufacturing, in particular with regards to pol analysis, clarification, evaporation and crystallisation rate of sucrose, factory capacity, scale formation, crystal shape, exhaustibility of massecuites, molasses purity and gelling of molasses. The authors concluded that gums can seriously reduce the efficiency of both raw sugar manufacturing and refining.

Why is it important to know the identities and location of gum-producing bacteria in the sugar manufacturing process?

The answer to this question is simple when referring back to the analogy of criminal profiling. If you know *who* the culprits are and *where* they are, attempts can be made to stop them from continuing with their crime. Microbial profiling follows the same principle; if you know *which* bacteria produce these undesirable gums and *where* in the process this phenomenon occurs, attempts can be made to develop a targeted approach to eliminate these microbes, preventing them from growing, reproducing and ultimately, using sucrose to produce gums.

Admittedly, many sugar technologists and industry experts will quite confidently declare that it is well known that *Leuconostoc* spp. are responsible for post-harvest cane deterioration and that it is the produced dextran which is the ‘gum' responsible for the processing problems. This statement is not incorrect, but it is not the full truth either. To explain the origin of this tendency to blame only *Leuconostoc* for post-harvest deterioration, one needs to look at the history of this subject.

As mentioned earlier, Van Tieghem (1878) isolated the microorganism responsible for gelification of sugar syrups and named it *Leuconostoc mesenteroides*. Hehre (1941) and Stacey (1942) confirmed this finding by obtaining dextran when growing *L. mesenteroides* on media containing sucrose. In 1947, McCleskey and co-workers reported on the characteristics of *L. mesenteroides* from cane juice. These authors evaluated 168 isolates and they observed four distinct ‘groups’ of bacteria according to colony types. These four groups of bacteria not only had distinctly different appearances, they also differed from one another with regards to fermentation reactions, amount of gum produced, gas and acid production, and in the pH and temperature required for growth. Yet these authors classified all these isolates as *L. mesenteroides* simply because they produced gum from sucrose and were able to ferment a pentose sugar (xylose or arabinose). The connection between gum (dextran) formation from sugar and blaming *Leuconostoc* as the only causative agent became customary; to illustrate, Lillehoj and co-workers (1984) published a paper on *Leuconostoc* spp. in sugarcane processing samples. These authors identified bacteria present in cane juice as *Leuconostoc* on two suppositions: (i) that the culture medium used to isolate the bacteria from cane juice (Rogosa *et al.*, 1951; Rogosa, 1970) was selective *only* for species of *Leuconostoc* and *Lactobacillus*, and (ii) that although both *Leuconostoc* spp. and *Lactobacillus* spp. can grow on a sucrose-based medium, only the *Leuconostoc* spp. would produce dextran. However, a study four years earlier on the microflora of sugarcane, cane juice and processing liquors (McNeil and Bond, 1980) pointed out that, “Leuconostoc *is not the only species likely to promote the slimes associated with juices and the ‘frogs’ spawn’ effects on mills*.” These authors further referred to a study by Perquin (1940), which implicated *Lactobacillus* spp. in dextran formation and *Bacillus subtilis* as levan (a fructose-type gum) producers. From their own work, McNeil and Bond (1980) reported that the majority of isolates from cane juice which produced gums from sucrose were species of either *Leuconostoc* or *Lactobacillus*, and

that it was very difficult to differentiate between the two genera. Even though there was ample evidence to show that *Leuconostoc* alone was not responsible for gum/dextran production, the majority of publications on the topic of post-harvest cane deterioration in years to follow only mentioned *Leuconostoc* as the causative agent (Antier, 1996; Godshall *et al.*, 2000; Solomon, 2000; Morel du Boil, 2001; Eggleston, 2002; Eggleston and Legendre, 2003; Eggleston *et al.*, 2001, 2004, 2009a).

In defence of the overwhelming blame of *Leuconostoc* for cane deterioration and dextran production, one may concede the following: (i) commercial dextran is produced by *L. mesenteroides* (Naessens *et al*, 2005) and (ii) the only way to identify bacteria in the early days (pre-1985: Woese *et al.*, 1985; Woese, 1987) was by the evaluation of their biochemical and physical properties (i.e. Gram-staining, colony morphology and growth requirements). However, due to the advances in molecular biology technologies and techniques and, more specifically, due to the ease of genotyping (i.e. examination of an organism's DNA and comparison with a reference strain) it is now possible to identify bacteria often at sub-species and even at strain level. Contrary to the work of Lillehoj and co-workers (1984) we now know that *Lactobacillus* spp. (and other bacteria such as *Streptococcus* sp. and *Weissella* sp.) are in fact able to produce dextran (and other gums) from sucrose (Kralj *et al.*, 2004; Bounaix *et al.*, 2010; Lakshmi Bhavani and Nisha, 2010; Palomba *et al.*, 2012; Paulo *et al.*, 2012). An MSc study by Willard (2012) on the (genotypic) identification of gum-producing micro-organisms from milled sugarcane revealed a diverse population of bacteria which were isolated: *Acinetobacter* spp., *Psychrobacter* sp., *Enhydrobacter aerosaccus*, *Enterobacteriaceae* spp., *Poryphory-monas* sp., *Weissella* sp., *Leuconostoc* spp., *Streptococcus* spp, *Bacillus* spp., *Microbacterium ginsengisoli*, *Micrococcus luteus* and *Propionibacterium acnes*. – notably, all of these bacteria were isolated from crushed sugarcane and were found to produce gums from sucrose.

Current methods applied to prevent microbially-produced gums and alleviate the effects of produced gums on sugarcane processing

Sugar factories generally strive to maintain a sound housekeeping regime, with physical cleaning of the factory, in order to reduce sucrose losses and gum production due to contaminating microorganisms; some factories will also apply a chemical microbicide as mill sanitation biocide. However, biocides are often ineffective; whether they are broad-spectrum (aimed at killing a group of microorganisms) or narrow-kill, they are not able to completely eradicate all microbial activity due to the low levels of added chemicals allowed by the American Food and Drug Administration (FDA). In addition, low dosages of biocides (especially the ones based on quaternary ammonium compounds) promote the development of resistance and immunity against them (Kulkarni, 1999). Resistance to biocides can also be a function of the formation of biofilms. All microorganisms can form biofilms upon adhesion to surfaces and a variety of different microbial species can be embedded in polysaccharide matrices, which act as penetration barrier against the active compounds in biocides, rendering them ineffective (Heinzel, 1998).

Another strategy adopted by some sugar factories is to treat the effect and not the cause of the problem by adding dextranase enzymes to factory streams in an attempt to reduce the dextran content. However, the application of dextranase enzymes has not been optimised and, as such, is not widely regarded as the solution to gum-related problems in sugarcane processing, as this also relies heavily on the assumption that dextran is the major fraction of the total gums present in processing streams. Factors contributing unfavourably to the potential

application of dextranase enzymes in the sugar industry include the uncertainty about which dextranase to use, the lack of an universal unit of dextranase activity which leads to confusion concerning dosages, and the price per unit of enzyme. In addition, the small market and low volume sales of dextranase enzymes compared to other industrial enzymes (such as amylases) have not encouraged research and development efforts into tailoring of dextranases to conditions specific to sugar factory process streams (Morel du Boil and Wienese, 2002; Eggleston *et al*., 2009b).

Current methods to prevent gum formation and subsequent processing problems, such as sucrose losses, are not adequate, whether they address the cause of the problem, or the effect thereof. Not one chemical microbicide and/or enzyme application has proven to be entirely successful in alleviating microbial contamination in sugar factories and the subsequent effects thereof. Furthermore, the initial source of microbial infection/contamination, from the sugarcane itself after harvesting, is currently not targeted as a first step in reducing sucrose losses and levels of gums in processing streams. Knowing the identities of the gum-producing microorganisms and their exact point of origin and subsequent locations in a sugar factory, will facilitate the development of tailor-made formulations designed to eradicate specific gum-producing microbes in sugarcane processing.

Microbial diversity profiling of gum-producing microorganisms in sugarcane processing – *how*?

It is acknowledged that the microbial diversity in sugar factories includes filamentous fungi, yeasts and bacteria. For this section, however, bacteria will be used as an example since gum-producing microorganisms regularly fall into this category. Traditional methods for the identification of bacterial genera and species required the evaluation of phenotypic profiles (i.e. observable characteristics of an organism). These phenotypic profiles include results obtained from Gram-staining, colony morphologies, growth requirements and enzymatic and/or metabolic activities; however, these characteristics are not static and can change with stress or evolution (Ochman *et al.*, 2005; Petti *et al.*, 2005). Advances in molecular biological techniques offered new opportunities for the analysis of the structure and species composition of microbial communities. As such, genotypic identification of microorganisms by 16S rRNA gene sequencing has emerged as a more objective, accurate and reliable method for bacterial identification, with the added capability of defining taxonomical relationships between bacteria (Clarridge, 2004).

The 16S rRNA gene sequence is approximately 1500 base pairs (bp) long and is composed of both variable and conserved regions. This gene is large enough, with sufficient interspecific polymorphisms (i.e. two or more clearly different phenotypes in the same population of species) to provide distinguishing and statistically valid data. The 16S rRNA gene sequence has been determined for a very large number of strains; GenBank (the largest database of nucleotide sequences) has over 20 million deposited sequences, of which over 90 000 are of the 16S rRNA gene. Consequently, there are many previously deposited sequences against which to compare the sequence of an unknown bacterial sample (Clarridge, 2004). Several other molecular typing techniques have been developed during the past decade for the identification and classification of bacteria at or near strain level, and similar methods are available for the identification of filamentous fungi and yeasts.

Conclusion

There is a need to understand the microbial diversity in sugarcane processing and, in particular, with regards to the microbial production of gums. This paper has highlighted the long history of (as yet) unsolved problems with gums in sugarcane processing. It has also underlined the importance of identifying gum-producing microorganisms instead of assuming that *Leuconostoc* sp. is the sole culprit responsible for sugarcane processing problems, and has given an example of how to identify gum-producing bacteria (16S rRNA gene sequencing). The purpose of microbial diversity profiling of gum-producers is to ultimately enable the development of a targeted approach to eliminate particular microorganisms (which have been confirmed as gum-producers) from specific locations in a sugar factory and relevant processing streams in order to reduce sucrose losses and the associated adverse effects of gums on cane processing. These are important factors to consider for the improvement of the economics of sugar production and sustainability of the sugar industry.

REFERENCES

Anon (1993). Laboratory studies on cane deterioration. Sugar Milling Research Institute Annual Report for 1992-1993: pp 5-6.

Antier P (1996). Microbiological control in a cane sugar mill: Implications on sugar quality and on losses. *Proc S Afr Sug Technol Ass* 70: 185-188.

Atkins PC and McCowage RJ (1984). Dextran – An overview of the Australian experience. *Proc Sug Proc Res Inst* 1984: 108-140.

Bounaix M-S, Robert H, Gabriel V, Morel S, Remaud-Siméon M, Gabriel B and Fontagné-Faucher C (2010). Characterisation of dextran producing *Weissella* strains isolated from sourdoughs and evidence of constitutive dextransucrase expression. *FEMS Microbiol Lett* 311(1): 18-26.

Cerutti de Guglielmone G, Diez O, Cárdenas G and Oliver G (2000). Sucrose utilisation and dextran production by *Leuconostoc mesenteroides* isolated from the sugar industry. *Sug J* 62(10): 36-41.

Clarridge J (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev* 17: 840-862.

Cuddihy Jr JA, Porro ME and Raiih JS (2001). The presence of total polysaccharides in sugar production and methods for reducing their negative effects. *J Am Soc Sug Cane Technol* 21: 73-91.

du Clou H and Walford SN (2012). Analytical study of gums found in the final molasses from six South African mills for the 2011/2012 milling season. *Proc S Afr Sug Technol Ass* 85: 295-311.

Eggleston G (2002). Deterioration of cane juice – sources and indicators. *Food Chem* 78: 99-107.

Eggleston G and Legendre B (2003). Mannitol and oligosaccharides: potential new criteria for determining cold tolerance in sugarcane varieties. *Food Chem* 80: 451-461.

Eggleston G, Legendre B and Richard C (2001). Effect of harvest method and storage time on cane deterioration. I: Ccane quality changes. *Int Sugar J* 103: 331-338.

Eggleston G, Legendre BL and Tew T (2004). Indicators of freeze-damaged sugarcane varieties which can predict processing problems. *Food Chem* 87: 119-133.

Eggleston G, Karr J, Parris A and Legendre B (2009a). A rapid biochemical test to assess postharvest deterioration of sugarcane and milled juice. *Sugar Tech* 11(2): 189-195.

Eggleston G, Monge A, Montes B and Stewart D (2009b). Application of dextranases in sugarcane factory: Overcoming practical problems. *Sugar Tech* 11(2): 135-141.

Godshall MA, Legendre BL, Richard C and Triche R (2000). Effect of harvest system on cane juice quality. *Proc Sug Proc Res Conf*, Porto, Portugal, pp 222-236.

Hehre EJ (1941). Production from sucrose of a serologically reactive polysaccharide by a sterile bacterial extract. *Science* 93: 237-238.

Heinzel M (1998). Phenomena of biocide resistance in microorganisms. *Int Biodeterior Biodegr* 41: 225-234.

Imrie FKE and Tilbury RH (1972). Polysaccharides in sugarcane and its products. *Sug Technol Rev* 1: 291-361.

Kralj S, van Geel-Schutten GH, Dondorff MMG, Kirsanovs S, van der Maarel MJ and Dijkhuizen L (2004). Glucan synthesis in the genus *Lactobacillus*: isolation and characterisation of six glucansucrases genes, enzymes and glucan products from six different strains. *Microbiology* 150(11): 3681-3690.

Kulkarni VM (1999). A novel method to evaluate efficacy of mill sanitation biocides: reducing sugar and titrable acidity analysis of final molasses. *Sugar Tech* 1(3): 54-62.

Lakshmi Bhavani A and Nisha J (2010). Dextran – The polysaccharide with versatile uses. *Int J Pharma Bio Sci* 1(4): 569-573.

Lillehoj EB, Clarke MA and Tsang WSC (1984). *Leuconostoc* in sugarcane processing samples. *Proc Sug Proc Res Inst* 1984: 141-151.

McCleskey CS, Faville LW and Barnett RO (1947). Characteristics of *Leuconostoc mesenteroides* from cane juice. *J Bacteriol* 54: 697-708.

McNeil KE and Bond JF (1980). The identification, enumeration and properties of microflora of sugarcane, cane juice and processing liquors. Technical Report No. 154 of the Sugar Research Institute, Mackay, Australia. 78 pp.

Morel du Boil PG (2001). Losses associated with post-harvest and pre-delivery conditions. *Proc Int Soc Sug Cane Technol* 24: 382-383.

Morel du Boil PG and Wienese S (2002). Enzymic reduction of dextran in process – Laboratory evaluation of dextranases. *Proc S Afr Sug Technol Ass* 76: 435-443.

Muller DA (2000). Criminal profiling – Real science or just wishful thinking? *Homicide Stud* 4(3): 234-264.

Naessens M, Cerdobbel A, Soetaert W and Vandamme EJ (2005). *Leuconostoc* dextransucrase and dextran: production, properties and applications. *J Chem Technol Biotechnol* 80: 845-860.

Ochman H, Lerat E and Daubin V (2005). Examining bacterial species under the spectre of gene transfer and exchange. *Proc Natl Acad Sci USA* 102: 6595-6599.

Palomba S, Cavella S, Torrieri E, Piccolo A, Mazzei P, Blaiotta G, Ventorino V and Pepe O (2012). Polyphasic screening, homopolysaccharide composition and viscoelastic behaviour of wheat sourdough from a *Leuconostoc lactis* and *Lactobacillus curvatus* exopolysaccharides producing starter culture. *Appl Environ Microbiol* 78(8): 2732-2747.

Pasteur L (1861). Sur la fermentation visquese et la fermentation butyrique (On the viscous fermentation and the butyrous fermentation). *Bull Soc Chim Paris* 11: 30-31.

Paulo EM, Vasconcelos MP, Oliveira IS, Affe HMdJ, Nascimento R, de Melo IS, Roque MEdA and de Assis SA (2012). An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with rapid confirmation. *Ciênc Tecnol Aliment* 32(4): 710-714.

Perquin LKC (1940). On the incidental occurrence of rod-shaped, dextran producing bacteria in a beet sugar factory. *Antonie Leeuwenhoek* 6: 227-249.

Petti CA, Polage CR and Schreckenberger P (2005). The role of 16S rRNA gene sequencing in identification of microorganisms misidentified by conventional methods. *J Clin Microbiol* 43(12): 6123-6125.

Ravnö AB and Purchase BS (2005). Dealing with dextran in the South African sugar industry. *Proc S Afr Sug Technol Ass* 79: 28-47.

Rogosa M (1970). Characters used in the classification of lactobacilli. *J Syst Bacteriol* 20: 519-533.

Rogosa M, Mitchell JA and Wiseman RF (1951). A selective medium for the isolation and enumeration of oral and fecal lactobacilli. *J Bacteriol* 62: 132-133.

Scheibler C (1874). Investigation on the nature of the gelatinous excretion (so-called frog's spawn) which is observed in production of beet-sugar juices. *Z Dtsch Zucker-Ind* 24: 309-335.

Solomon S (2000). Post-harvest cane deterioration and its milling consequences. *Sugar Tech* 2(1,2): 1-18.

Solomon S, Shrivastava AK, Singh I, Sowani A and Prajapati CP (2008). An assessment of post-harvest sucrose losses in sugarcane billets under sub-tropical conditions. *Int Sug J* 110(1312): 236-241.

Stacey M (1942). Enzymatic production of bacterial polysaccharides. *Nature* 149: 639.

Van Tieghem P (1878). Sur la gomme de sucrerie (On sugar-mill gum). *Ann Sci Natur Bot* 7: 180-203.

Vettori MHPB, Blanco KC, Cortezi M, de Lima CJB and Contiero J (2012). Dextran: Effect of process parameters on production, purification and molecular weight and recent applications. *Diálogos Ciência* 31: 171-186.

Willard K (2012). Investigation of exopolysaccharides producing bacteria isolated from milled sugarcane. MSc Thesis (Plant Biotechnology), University of Stellenbosch, South Africa. 71 pp.

Woese CR (1987). Bacterial evolution. *Microbiol Rev* 51: 221-271.

Woese CR, Stackebrandt E, Macke TJ and Fox GE (1985). A phylogenetic definition of the major eubacterial taxa. *Syst Appl Microbiol* 6: 143-151.