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Identification of Dextran and Sludge–Producing Bacteria in Sugar Cane Juices Using Polymerase Chain Reaction

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The microbial contamination due to cane sugar transition to the mills is one of the most important factors in increasing sugar lesions in the factory. This study was aimed to isolate dextran-producing bacteria and determine their genus and species. It is a descriptive cross-sectional study which was conducted in year 2014. For this purpose, 200 samples were collected from sugarcane syrups, and then cultured as pourplate and surface. The bacteria were counted and the DNA extracted from the purified bacteria according to the kit protocol. Then, determination of the genus and species of dextran-producing bacteria was performed by polymerase chain reaction (PCR) using specific primers. Data obtained from biochemical, microbial and PCR showed that around 80 strains of *leuconostoc* have been detected in samples. The results of this study indicate that *leuconostoc mesenteroides* is the main factor in the production of dextran in sugarcane and beet manufactories. The above mentioned contamination sources can be minimized by reducing the transfer time of burned sugarcane to the factory, as well as the regular physical and chemical washing of the mills.

Keywords: Sugarcane, dextran, *leuconostoc*, Polymerase chain reaction (PCR).

The activity of microorganisms in sugarcane begins through the soil and putrescent plants. Therefore, sugarcane -extracted juice contains a large number of microorganisms. One milliliter of such extract contains a billion bacteria and a million molds and yeasts[1]. Bacteria commonly found on green sugarcane leaves include *Xanthomonas*, *Lactobacillus*, *Flavobacterium*, *Corynebacterium*, *Bacillus*, *Pseudomonas*, *Enterobacter* and *Ervinia*. Some of these bacteria are pathogenic to the plants. Following the previous studies, more than 50 types of bacteria have been isolated from green sugarcane. These studies also reported 17 different microorganisms from burned sugarcane among which, *leuconostoc mesenteroides*, polysaccharides-producing bacteria such as *saccharomyces*, Soil bacteria such as *Pseudomonas* and *Bacillus cereus*, *Actinomycetes* and acid-producing *streptomycetes* bacteria have been identified[2]. *leuconostoc* strain has the ability to produce sludge substances in the factory. Although green sugarcane has usually no contamination with *leuconostoc*, however, breaking the growing cane stems leads to entrance of the bacterium into the

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cane tissues. Addionally, burning of sugar cane destroys the wax-surface protector of the stem and produces the cracks in the outer crust and burns the storage tissues underneath. These events eventually lead to collapse of the stem and secretion of juice outside. This produce a good environment for the microorganism growth[1,3]. Microorganisms, especially *leuconostoc* can grow on burned sugarcane. Following two hours of burning, the bacteria growth on corn stalks and degrades the sugar. Dextran and acid can be produced in this way[1-4]. Produced dextran can create some problems in the milling process and refinery the syrup. Growing body of evidences have been shown that stopping of mill for more than 24 hours, increase the dextran levels up to the 34%[4]. *Leuconostoc mesenteroides* is a bacterium capable to produce viscous materials such as dextran, which compete with other bacteria in canesugar. This bacterium has capable to produce invertase enzyme and sucrose-degrading enzyme[1, 5]. Therefore, the purpose of this study was to identify the genus and species of *Leuconostoc* isolated from juice-extracted from cane sugar using molecular detection system.

MATERIAL AND METHODS

Sampling

In this study, 200 samples of extracted syrup were randomly collected from 5 mills of Dehkhoda mills industry and stored in sterile containers and transferred to the quality control laboratory. The PH, temperature, percentage of sugar, brix and extraction purity was recorded immediately.

Culture of bacteria

To prepare a dilution from the liquid samples, 1 ml of the specimen was pipetted into a sterile tube containing 9 ml normal sterile salin after homogenization of the samples. The contents of the tubes were mixed with the shaker until obtaining a homogeneous solution. Dilutions 10^{-1} to 10^{-7} were prepared and then cultured in purplate and Surface culture and incubated for 48 to 72 hours at 37 $^{\circ}$C. The growing bacteria were purified using a pittido dextrose agar culture media containing 20% sucrose. The oxidase and catalase tests as well as other physicochemical and linear culture methods were also obtained in this way.

Storage and extraction

For short-term storage, the purified specimens were cultivated in tubes containing agar nitrate and broth nitrite while for prolonged storage, samples were cultivated in a Slim Milk environment and maintained at -20 $^{\circ}$C until use. The genomic DNA was extracted using DNA extraction kit (Qiagen, USA) based on its manufactured protocol.

Polymerase chain reaction (PCR)

In order to optimize the polymerase chain reaction, a standard strain of *Leuconostoc mesenteroides* (PTCC-1591) was purchased from the microbial bank of Pasteur Institute of Iran and was used to determine the sensitivity of the PCR method. The genomic sequence of 16srDNA was used to trace the bacteria. The specific primers were designed using the Oligo 7 software. The forward and reverse primer sequences were as follow: LeuF:GGAAAGGTGCTTGCACCTTTCAAG and LeuR:TTTGTCTCCGAAGAGAACA. Polymerase chain reaction was carried out final volume of 20 µl containing 3 µl dNTP Mix, 5 µl PCR buffer 10X, 1 µl mgcl2 0.5 µl of each primer, 0.3 µl of Taq Polymerase enzyme, 2.5 µl sterilized distilled water, and finally 4.5 µl extracted DNA was added to each test tube. Thermocycler was programed as initial denaturation at 95 $^{\circ}$C, followed by 30 cycles of 94°C; 1 minute, 63 $^{\circ}$C; 1 minute, and 72 $^{\circ}$C; 1 minute and final extension at 72 $^{\circ}$C for 5 minutes. To determine the size of the PCR bands, electrophoresis was performed on 1.5% agarose gel.

RESULTS

As shown in Table1, microbial counting in the milling section show a high rate of juice contamination in different parts of juice mills, most of them including aerobic bacteria, mesophilic, acid generators and sludge producers. Following the microbial and biochemical tests, 80 types of *Leuconostoc* strains were isolated (40%). Molecular analysis revealed that all isolates show 970bp band in PCR-electrophoresis (Fig. 1) and all of them were diagnosed as *Leuconostoc mesenteroides*.

DISCUSSION

High rate of juice contamination was observed in the various parts of the mill. The contamination was mostly connected to aerobic mesophilic bacteria, acid producing and sludge producing. This could be due to a high interval time between burning of cane and its transfer into the factory or a low level of hygiene and washing in the factory. The present germs in the rolls and porosity of the mills surfaces can be continuously multiplied and increase their number in the mill-extracted syrup. Therefore, contamination in the mill unit can increase the contamination of input sugar cane into the factory.

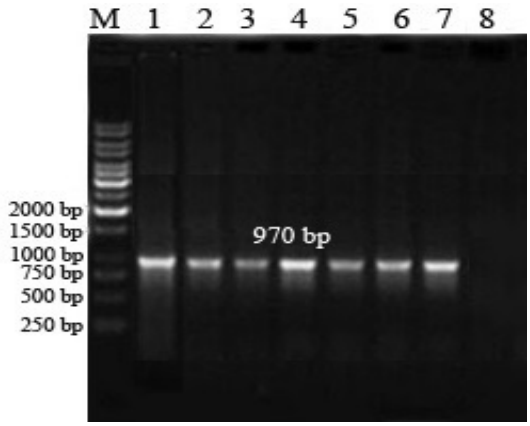

Fig. 1. PCR-electrophoresis. M: 1kb size marker, 1: positive control of *Leuconostoc mesenteroides* PTCC-1591 (970bp), 2-7: isolated from *Leuconostoc* and 8: negative control

The obtained data showed that the syrup contamination rate was reduced following mill washing with boiling water or steam. Previously, Parcha *et al*. emphasized the need for using physical washers with ability to produce high pressure water at the factory[5].

Physical washing using high pressure of water and steam is one of the old and the most effective physical methods in the cane industry to reduce the contamination from the mill. In 1999 and 2003, Compen and Moyesha reported physical methods such as washing and steaming are effective while some others like such as the use of UV rays are less efficient. Every 8h steam cleaning coupled with steam jetting in slots and chain connections can be used to reduce pollution up to 60%, and chemicals can be used for remaining[6,7], which in consistent with the data obtained in present study.

Slime-causing bacteria are microorganisms that form shiny capsules when they grow on sucrose. These bacteria include Bacillus, Streptococcus, Lactobacillus and *Leuconostoc*. Most of the bacteria in the factories are, slag- and acid-producing bacteria which were usually enter into the factory through the soil's farm and are normal flora (common microorganisms) of sugarcane in the farm[7,8].

Five to sixteen percent of soil bacteria can synthesize sucrose, a high molecular weight polymer, and these bacteria are also considered to

Table 1. The obtained data from bacterial counting

Aerobic a mesophilic bacteria Cfu/ml	Slime-causing bacteria Cfu/ml		Aerobic a mesophilic bacteria Cfu/ml	Slime-causing bacteria Cfu/ml	Mold and yeast	Purity	Pol	Brix	Juice Extract
10×10^3	14×10^3	After	22×10^5	20×10^5	14×10^2	79.76	13.32	16.70	First
22×10^3	38×10^3	steam	42×10^5	26×10^5	38×10^2	78.16	10.52	13.46	Mix
28×10^3	26×10^3	washing	18×10^5	92×10^5	26×10^2	59.9	1.24	2.07	Last
63×10^3	22×10^3		18×10^5	48×10^5	22×10^2	80.13	13.43	16.76	First
18×10^3	62×10^3		40×10^5	12×10^5	24×10^2	78.47	10.57	13.47	Mix
32×10^3	84×10^3		20×10^5	36×10^5	70×10^2	60.3	1.20	1.99	Last
12×10^3	34×10^3		16×10^6	78×10^5	10×10^2	82.46	13.73	16.65	First
56×10^3	76×10^3		10×10^5	64×10^4	40×10^2	80.87	10.61	13.12	Mix
82×10^3	28×10^3		70×10^5	44×10^6	62×10^2	61.08	1.13	1.85	Last
10×10^3	58×10^3		76×10^5	18×10^4	66×10^2	86.78	13.85	15.96	First
76×10^3	36×10^3		18×10^5	90×10^5	34×10^2	85.65	10.27	11.99	Mix
42×10^3	24×10^3		20×10^6	88×10^5	80×10^2	59.89	1.06	1.77	Last

be the most important bacteria in bush and sugar cane factories[9]. Several studies on sugar beet plants have reported that different soil bacteria such as Bacillus, Lactobacillus, and *Leuconostoc* as the major genera identified in sugar beet plants. All of these bacteria belong to the family of Lactic acid bacteria[9,10,11]. *Leuconostoc* bacteria are the epiphytic bacteria that are widely distributed in natural environments. Jerry et al. (1961) are the first researchers who reported an article about the production of dextran from sucrose by different strains of the *Leuconostoc*[12]. In another study, Tannel et al. (1954) reported the production of dextran by sex strains of *Leuconostoc*, and also attributed the slurry production to sugarcane plants[12, 13]. The results of these studies are in agreement with the present study. Dextran-producing bacteria synthesize dextrose from sucrose under certain conditions like low amount of glucose[13]. Studies have shown that *Leuconostoc mesenteroides* converts glucoses which were produced during sucrose hydrolysis to dextran. The produced fructose will be converted into the manitol polyhydric alcohol, which is usually hard to use. Also, some *Leuconostoc* strains have the ability to produce polymers other than dextran, such as Alteran and Levan[14].

Therefore, the high contamination rate of this bacterium in sugar cane causes the synthesis of dextran, starch, levan and other polyester compounds, eventually leads to produce problems like an increasing unknown sugary lesions, in such condition, the amount of sucrose lesions becomes 1.9 times more than the concentration of dextran[1]. Additionally, increasing of the syrup turbidity prevents the sedimentation of suspended particles and, causes disruptions in the refinement so increased syrup viscosity, incomplete refinement and reduced efficiency and prolongation of the crystallization period. The latter one results in the loss of sugar during centrifugation[1,14]. Besides, the long-term crystallization time at lower temperatures is more understandable, as it results in the production of low-grade baking. Baking desserts are hardly centrifuged and a lot of sugar enters into the final molasses. Also, the magma made from this raw sugar is not suitable for the production of commercial sugar. Ultimately, high levels of dextran are accumulated in syrups and baking, which reduces the ability to extract sugar and causes problems in raw sugar storage. The two major groups of dextran-producing bacteria are from mill-extracted juice; *Leuconostoc mesenteroides* and *Leuconostoc dextranicum*, but only *Leuconostoc mesenteroides* has been found in contaminated sugar products (1.13 and 14). The isolated dextran from the sugarcane extract initially found as a linear polymer with more than 90% á[1,6] connections, although other types of dextran have different connections[13,14].

One of the other disadvantages of the dextran polymer is false increased in value of purity of syrup and calculating the amount of sugar extracted from it. As an example, every 333 ppm dextran in sugar cane and raw sugar extract falsely increase the purity equal to 0.15 ° (1) which causes a high level of error due to the large scale of work in sugar cane industry.

CONCLUSION

As syrup contamination can influence the amount of sugar lost, which was eventually increase the production costs, it is necessary to cope with such microbial contamination by applying the strategies to prevent stem stings from being slaughtered, reducing the interval time between the burning of sugar cane and its transfer to the factory, reducing the number of mill stops, and regular physical and chemical washing of mills.

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REFERENCES

1. Chen JCP, Chou CC.(1993) Cane sugar handbook: a manual for cane sugar manufacturers and their chemists. John Wiley and Sons, Inc.
2. Chung C. (2000). Handbook of sugar refining, A manual for the design and operation of sugar refining facilities, John Wiley & Sons, New York, Chapter28.
3. Broadnent JR et al. Biochemistry, genetics and application of exopolysaccharide production in streptococcus thermophilus: A review. *Journal*

of Dairy Science., 2003; **86**; 407-423.

4. Solomon S, Shirvastava A. post-harvest biological losses of soucrose by application of organo sulfure based biocide during milling of cane. Indian Institute of sugarcane research, Lucknow -226 002, India. Proc. 2005; ISSCT.25.
5. Purchae BS. Losses caused by micro-organisms. *International Society of Sugar Cane Technologists.,* 2001; **24**: 379-390.
6. Kampen WH, DF Day. (2003). Organic Acids(+). Annual meeting ASCT, Louisiana Division. Baton Roge, La. Feb 4-5.
7. Moutia M, Dookun. Evolution of surface sterilization and hot water treatment on bacteria contaminants in bud cultures of sugarcane. *Experimental agriculture.,* 1999; **35**: 365-374.
8. Setare M and Javaherdashti R. Assessment and control of MIC in sugarcane factory. *Material and corrosion.,* 2003; **54**(4): 256-263.
9. Beddie, D; Isles, N; Wirth, T; Pollach, G; Hein, W. Successful Application points to control Bacteria infections throughout sugar factories using Beta Acid. Poster presentation on the occasion of the SPRL conference in Atlanta, Georgia, USA, 2004; April 4-7.
10. Belami M, Mekkaoui A.K, Tantaoui-Elaraki A. Saccharolytic bacteria in beet juices, *International Sugar Journal,* 1991; **93**: 210-212.
11. Bugbee WM, Cole DF, Nielsoen G. Microflora and invert sugar in juice from healthy tissue of stored sugarebeets. *Applied Microbiology,* 1975; **29**: 780-781.
12. Jerry VM and Colmer RA. Selective medium for Leuconostoc detection, 1961; 18100-1011.
13. Thunell RK. Taxonomy of the Leuconostoc. *Journal of Dairy Scienve,* 1995; **78**: 2514-2522.
14. Holt SM et al. Characterization of dexteran producing Leuconostoc strains. *Letters in Applied Microbiology.,* 2001; **32**: 185-189.