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Incidence of *Cronobacter sakazakii* in Dairy-based Desserts

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ABSTRACT

Cronobacter sakazakii is one of emerging foodborne pathogens around the world. A total of 90 dairy-based desserts samples (ice cream, Muhallabia and rice pudding) were examined for detecting *C. sakazakii*. All samples were submitted for bacteriological examination and confirmed by molecular identification using 16S rRNA gene for *C. sakazakii*. The bacteriological and molecular examination revealed that the incidence of occurrence of *C. sakazakii* was 5.55% from the total dairy-based desserts samples, the highest percentage occurred in rice pudding samples (10%), while the incidence of *C. sakazakii* in ice cream and Muhallabia were 3.33% for each type. The results pointed out that high risk for human may occur by contaminated dairy-based desserts. The hygienic precautions must be taken during the processing of these types of products.

Introduction

Cronobacter sakazakii has been previously known as *Enterobacter sakazakii*. It is Gram-negative bacteria, motile peritrichous, rod-shaped, non-spore forming bacteria belonging to the family Enterobacteriacea. In addition, this bacteria is typically facultative anaerobic, oxidase-negative, catalase-positive, it also produce a yellow pigment (Iversen *et al*., 2007).

C. sakazakii is opportunistic pathogen associated with fatal neonatal infections, including meningitis, septicemia, sepsis and necrotizing enterocolitis (NEC), in addition, the mortality rates associated with Cronobacter infection have been broadly reported from 33 to 80% in infants (Nazarowec-White and Faber, 1997; Van Acker *et al*., 2001; Lehner *et al*., 2004; Stephan *et al*., 2007; Hunter and Bean ,2013). Beside the infant's infection, the infections also occur in older children and adults and it cause serious infections as septicemia, pneumonia, osteomyelitis, wound infection and splenic abscesses (Healy *et al*., 2010).

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C. sakazakii is recognized worldwide as emerging foodborne pathogens. Although, the nature reservoir of this bacterium is still unknown. It could be isolated from a wide variety of food as cereal foods (Chap *et al*., 2009), fruits and vegetables (Jung and Park, 2006). It also detected in food from animal origin as milk and milk products like cheese, dried milk, infant formula and ice cream (Friedemann, 2007; El-Shall, 2013; El-Gamal *et al*., 2013; Saad and Amin, 2014). Moreover, there is no record about the presence of *C. sakazakii* in popular dairy-based desserts sold in Egypt. The contamination of the products caused by poor raw materials used and poor handling during manufacture processing, packaging and storage.

Therefore, this work aimed to investigate the incidence of *C. sakazakii* in most popular dairy-based desserts in Egypt using conventional microbiological and molecular methods.

Materials and methods

Samples and study area

A total of 90 random samples of some dairy-based desserts including: ice cream, Muhallabia and rice pudding were been

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collected from different dairy shops and dairy desserts shops in Assiut City, Egypt, in clean, dry and sterile containers, while ice cream samples were taken in the ice boxes. These samples were transferred to the laboratory as soon as possible to be examined.

Preparation of samples

Twenty five grams from each sample were thoroughly mixed before being examined according to A.P.H.A. (2004).

Isolation of C. sakazakii (Kim et al., 2011)

Pre-enrichment procedure

The samples were pre-enriched by mixing 25 ml/g sample with Buffered peptone water (BPW). Mixed well and incubated at 37ºC for 24.0±2.0 h.

Enrichment procedure

Ten ml from incubated pre-enrichment were inoculated into Enterobactereace Enrichment Broth (EEB). Mix well and incubated at 37ºC for 24.0±2.0 h.

Plating on selective agar media

A loopful of incubated broth was subcultured into the surface of Chromogenic Cronobacter Isolation Agar (mDFI) (modified Druggan, Forsythe and Iversen agar) (Oxoid Ltd., Basingstoke, United Kingdom) followed by incubation at 37°C for 24 h.

Typical colonies appear as blue/green, which were transferred to Trypticase Soy Agar (TSA) and incubated at 37°C for 48 h. Colonies appeared as yellow pigmented.

Identification of C. sakazakii

Biochemical tests

Identification of the isolated *C. sakazakii* was done according to Iversen *et al*. (2008) by biochemical testing.

Polymerase chain reaction (PCR)

The suspected bacterial colonies (5 strains) and the reference strain were subcultured onto nutrient broth and incubated overnight at 37°C for DNA extraction using Patho Gene-spin TM DNA/RNA Extraction kit (ISO 9001/14001) according to manufacturer instruction. The extracted DNA was stored at -20°C.

The 16S rRNA gene was amplified using Esakf (5' GCT YTG CTG ACG AGTGGC GG 3') and Esakr (5' ATC TCT GCA GGA TTCTCT GG 3') primer pairs (Applied Biosystem, USA) (Angelika and Roger, 2004).

DNA amplification was performed in final volume 25 µl; 12.5 µl of 2X PCR master mix (Green Master, Promega, USA), 150 ng of the DNA template, 0.5 µM of each primer and up to 25 µl nuclease free water were mixed in a PCR tube. The amplification was performed in a programmable heating block (Gradient Thermal Cycler, Veriti Applied Biosystem, USA) at 94°C for two mins, followed by 30 cycles were run under the following conditions; denaturation at 94°C for 30s, annealing at 60°C for 60s and extension at 72°C for 90s. After final cycle the preparations were kept for 5 mins at 72°C as final extension.

PCR products were electrophoresed in 1% agarose gel (GX 040.90, Gen AGarose, L.E., Standard DNA /RNA agarose, Molecular Biology Grade, Inno–Train Diagnostic, D–61476, Kronberg/Taunus) Containing Ethidium bromide as 1µl /ml electrophoresis buffer at 100 volts for 60 min. Using 100 bp DNA–ladder in (SCiE–PLAS, HU 10, 5636, UK). The result obtained through High performance ultraviolet Trans illuminator, (UV, INC, UK). The bands of the PCR products containing the positive DNA sequence of 929 bp for 16S rRNA gene were analyzed using DOC–It®LS, Image acquisition–software, (Biodoc Analyzer, Biometra, Germany).

Results

The incidence of *C. sakazakii* was 5.55% from 90 samples of dairy-based desserts. One sample from both ice cream and Muhallabia contained this bacterium in a percentage of 3.33 for each type, while, three samples from the rice pudding samples contained the same bacteria in a percentage of 10%. (Table 1)

Results of molecular identification was shown in Fig. 1.

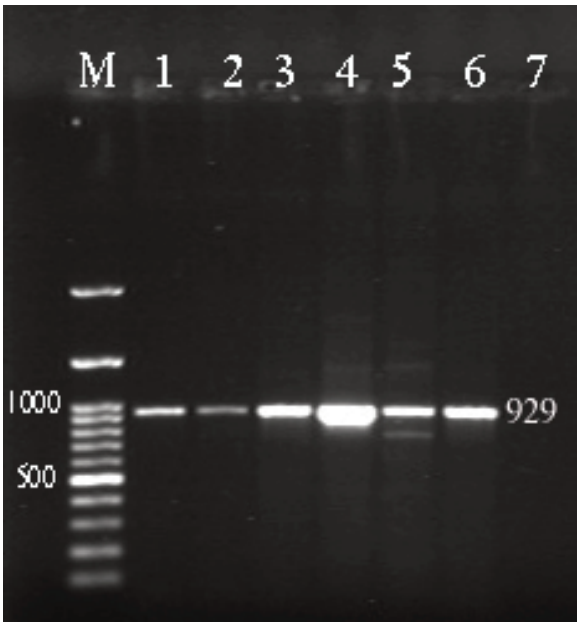

Fig.1. PCR products of amplified 16S rRNA of *C. sakazakii* visualized on agarose gel electrophoresis. Lane (M) DNA ladder 100 bp, lanes (1-5) positive with specific bands at 929 bp, lane (6) positive control and lane (7) negative control.

Table 1. Incidence of *C. sakazakii* in the examined dairy-based dessert samples.

Examined samples	No. of examined samples	Positive samples	
		No.	%
Ice cream	30	1	3.33
Muhallabia	30	1	3.33
Rice pudding	30	3	10
Total	90	5	5.55

Discussion

In recent years, dairy-based desserts have been increased rapidly in the marketplace. Dairy-based desserts can be manufactured by using fresh milk (skim or full fat) and/or milk powder. Flavors, colors and sweeteners may be added along with thickening agents to improve texture as starch. Other additives used as grains like rice and different types of nuts. The microbial contamination of these products is extremely varied, reflecting the ingredients nature of the products and variation of heat treatment methods used in the manufacture (Food Standards Australia New Zealand, 2006).

In this study, the identification of the organism was based on using the biochemical tests, the results were confirmed using conventional PCR. Gene 16S rRNA is the most common gene that is used for identification of bacteria including *C. sakazakii* (Angelika and Roger, 2004).

In the present study, the incidence of *C. sakazakii* was 5.55%from 90 samples of dairy-based desserts. One sample contained this bacteria in percentage of 3.33 in ice cream and Muhallabia, while, three samples (10%) of the rice pudding samples contained the same bacteria (Table 1).

The incidence of *C. sakazakii* in ice cream was nearly similar to the result obtained by El-Gamal *et al.* (2013), they examined 25 ice cream samples for presence of *C. sakazakii* and they found that only one sample (4%) carried the organism.

The highest incidence of *C. sakazakii* in rice pudding may be attributed to contaminated milk used in product manufacturing or due to post pasteurization and post processing contamination. Furthermore, the poor quality of the rice used in the product may be the source of contamination, as *C. sakazakii* could be isolated from the cereal foods (Chap *et al.*, 2009).

Conclusion

The most popular dairy-based desserts available to the consumer may have *C. sakazakii* contamination. So, restricted precautions must be taken during manufacture, handling and storage of these products. In addition, ingredients of high quality must be used to avoid any contamination by *C. sakazakii*.

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