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Wet heat treatment of Cronobacter sakazakii and detection of viable cells using RT-PCR and Propidium monoazide for distinction between dead and viable cells

Nielsen, Martin Thorup; Svendsen, Carina; Thorsen, Line; Jakobsen, Mogens

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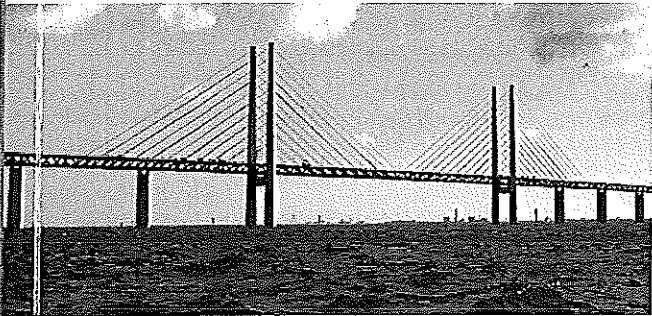
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.D2.15	Sondergaard T	PEB1.30	Sutherland JP	PEA2.18	Thorsen Line	PEA1.70
:C1.44	Song EA	PEB2.10		PEB2.39	Thorsen Line	PEB1.32
:D2.41	Song KW	PEC1.42		PSA1.01	Thorup Cohn M	PEB2.21
:A1.07	Sood R	PEB1.17	Sutyak, KE	PSA2.06	Thrane U	PEA2.44
A2.05	Soumaya Messaoudi	PEA1.33	Suzzi G	PEA1.56	Thuault D	PEC1.81
:C1.65	Spaziani M	PEB2.17	Suzzi G	PEC1.78		PEC1.82
A1.59	Speybroeck N	PEC1.30	Svendsen C	PED2.50		PEC1.103
:A2.41	Stabler R	PEB2.32	Svensson B	PEB2.06	Timan ADJ	PEB1.06
A2.46	Stabler R	PEB2.38	Svensson L	PEC1.92	Timke M	PEA2.29
D2.61	Stals A	PEC2.15	Sweeney T	PED1.01	Todorov S	PEA2.14
B1.31	Stals A	PEC2.48	Szlavik, Julie	PSD2.03	Todorov SD	PEA2.08
A2.03	Stamatiou A	PEC1.72	Söderholm Henna	PEB2.60	Todorov SD	PED2.48
B2.23	Stampelou I	PEC2.55	Söderholm, H	PSB2.01	Todorov Svetoslav	PEA2.23
A1.47	Stastkova Zora	PEC1.10	Sørensen G	PEC2.01	Tofalo Rosanna	PEA1.56
D2.24	Staufenbiel Anja	PEA2.06	Sørensen KI	PEA1.40		PEC1.78
D2.11	Stecchini M	PEB2.17	Sørensen LM	PEA1.10	Tomasevic I	PED2.49
B2.30	Stefanelli E	PEB2.37	Sørensen S	PEB1.21	Tomic N	PED2.49
B2.47	Stephan R	PED1.15	Sørensen SJ	PEE2.14	Tononi P	PEB2.37
B2.50	Stephan, R	PSB1.02,	Sørensen, SJ	PSE1.03	Torabi P	PEB1.27
C2.46	Stessl B	PEC1.95	Tabanelli G	PEA1.30	Torriani S	PEA1.29
B1.06		PEC1.98		PEE2.08		PEA1.30
D2.21		PEC1.99	Tahar A	PED2.01		PEB2.37
C1.63	Stevens G	PEE2.02	Taivosalo A	PEA1.15	Torrieri E	PED2.31
A1.17	Stevens, M	PSE1.01,	Tajbakhsh M	PEB1.27	Toyofuku Hajime	PEC2.18
C2.47	Steyn Cató	PED2.02	Talon R	PEA1.04	Tran-Dinh N	PEB2.56
:J1.35		PED2.03	Talon R	PEB2.03	Traversa A	PEA2.42
:J2.16	Stjepanovic Aleksandra	PEA1.17	Taminato F	PEB2.23	Trivedi Krina	PEA1.09
A1.48	Stonsaovapak S	PED2.11	Tanfani F	PEB2.17	Trivedi Krina	PEB1.05
:J2.55	Stonsaovapak Siriporn	PEA1.08	Tango N	PEA1.68	Troianiello GD	PEA1.75
:A2.44	Storm C	PED1.23	Tanner R	PEB1.03	Tromp, S-O	PSC2.02
:J2.37	Storm Ida Marie		Tanner, S	PSE1.01,	Truchado P	PED2.42
:J2.49	Lindhardt Drejer	PEA2.44	Tano-Debrah K	PEA1.36	Truelstrup Hansen L	PED2.07
:J1.27	Strachan Norval	PEC2.29	Tano-Debrah K	PEA1.37	Tsai SM	PEA1.07
:J2.06	Strachan, Norval	PSC2.06	Taoukis P	PEC1.87	Tsakalidou E	PEA1.11
:J2.04	Strand Å	PEA1.57	Taoukis P	PEC1.96	Tsevdou Maria	PEC1.87
31.11		PED1.31	Tarczynska AS	PEC1.27	Tsevdou Maria	PEC1.96
:J1.84	Straver J	PEC2.37	Tarczynska AS	PEC1.28	Tsironi Theofania	PEC1.87
:J2.52	Strini A	PED1.16	Tasara, Taurai	PSB1.02	Tudela, JA	PSD1.04
:J1.51	Strydom Arny	PEC1.23	Tassoni A	PEA1.68	Tungtrakul P	PEA1.08
:J1.27	Studeníèová A	PEC1.14	Tassou C	PEC1.53	Turhan Ö	PEA2.16
:J1.31	Stulova I,	PEC1.24	Team RELU	PEC2.29	Turpin Williams	PEA1.44
:J1.33	Stulova Ifina	PEA1.15	Teixeira JA	PEA1.26	Uhrig S	PEE2.10
:J1.81	Stüber E.	PED1.11	Teixeira P	PEA2.41	Urbán Carrillo G	PEC2.38
:J1.82	Stüber Elisabeth	PEB1.14	Teixeira P	PEB1.31	Uyttendaele M	PEC1.30
.103	Suba S	PEB1.17	Tekin E	PEA1.47		PEC1.46
:J2.48	Subires Alicia	PEB2.28	Tempelaars M	PEB2.29		PEB2.07
:J2.44	Subires, Alicia	PSB2.03,	Tenehaus F	PEC2.37		PEC2.07
:J2.62	Sudharshana MR	PED1.10	Ter Beek A	PEB2.04		PEC2.15
.1.06	Sugita-Konishi Y	PEB1.13	Tersteeg-Zijderveld MHG	PEB1.06		PEC2.18
:J2.21	Suhajda Á	PED1.19	Theron MM	PEC1.51		PEC2.35
:J2.24	Suhajda Á	PED1.20	Thevenot-Sergentet D	PEC1.22		PEC2.36
:J2.11	Susítha K	PED2.24	Thiel S	PEC2.23		PEC2.48
:J2.13	Sutherland Jane P	PEC2.28	Thierry A	PEE1.01		PEC2.56
:J2.14			Thierry, A	PSA1.06		PED2.49
:J2.01,			*Thorsen L	PED2.50		

- * PED2.50 Wet heat treatment of *Cronobacter sakazakii* and detection of viable cells using RT-PCR and Propidium monoazide for distinction between dead and viable cells
 Martin Thorup Nielsen (1), C Svendsen (1), L Thorsen (1), M Jakobsen (1)
 (1) KU-LIFE, Denmark

The food borne pathogen *Cronobacter sakazakii*, previous known as *Enterobacter sakazakii*, is associated with powdered infant formulas (PIF). Because *C. sakazakii* is related to outbreaks of meningitis, septicaemia and necrotizing enterocolitis in neonates it is of great interest to obtain a better understanding of the heat treatment which is needed to inactivate *C. sakazakii*. We propose a new way of evaluating heat treatment by use of Propidium Monoazide RT-PCR. Propidium Monoazide (PMA) enters the nucleus of bacterial cells with damaged membranes (non-viable). Upon light exposure PMA is converted into a highly unstable nitrene intermediate with a high affinity for DNA. The nitrene intermediate is quickly covalently bound to the DNA present. After light exposure DNA is extracted from viable and non-viable cells using commercially available DNA extraction kits. PMA bound DNA will thus be extracted together with the non-PMA bound DNA. DNA, which is bound to PMA, is no longer capable of interacting in PCR reactions and it is therefore possible to use quantitative RT-PCR to determine the number of viable cells. In the present study a primer set specific *C. sakazakii* was used in the RT-PCR. The PMA RT-PCR method was used to determine D-values and a z-value for *C. sakazakii* cells after heat treatment. For verification purposes CFU data was used. Thermal inactivation of *C. sakazakii* DSM 4485^T was conducted in triplicates, D-values and a z-value was obtained. Heat treatment was carried out in Erlenmeyer flasks containing BHI-broth preheated to either 52 °C, 54 °C 58 °C and at 60 °C. The corresponding D-values were found to be 46.5 min, 10.56 min, 3 min and 1.6 min as obtained from CFU determinations. For the PMA RT-PCR determination of viable cells 1 mL of heat treated *C. sakazakii* cells was centrifuged (14.000g) for 5 min at 5 °C and the supernatant was discarded. The pellet was suspended in 0.55 mM PMA for 10 min in the dark at 5 °C, with 5 sec vortexing every 2 min. The sample was hereafter exposed to light (650W) for 3 min at a distance to the light source of approximately 20 cm. The RT-PCR data obtained were used to evaluate the validity of the results obtained by CFU determinations. This is the first time PMA RT-PCR is used distinction of dead and viable cells of *C. sakazakii*.

- PED2.51 *L. monocytogenes* is gradually inactivated in three processed cheese analogs stored under proper refrigeration but grows under severe temperature abuse.
 Apostolos Angelidis (1), S Georgiadou (1), D Papageorgiou (1)
 (1) Aristotle University of Thessaloniki / School of Veterinary Medicine, Greece

The behaviour of *Listeria monocytogenes* was studied in three processed cheese analogs (PCAs) that were inoculated to contain less than 100, ca. 5×10^3 and ca. 5×10^5 CFU of a cocktail of three *L. monocytogenes* strains per g. The inoculated products were aseptically packaged under vacuum to mimic their respective market package conditions and stored at 4, 12 and 22°C. Based on their physicochemical characteristics (a_w and pH) and their shelf life neither of the PCAs can be automatically classified as products unable to support the growth of *L. monocytogenes* according to the definition given in Annex I of the Commission Regulation (EC) 2073/2005 and its amendment by Commission Regulation (EC) 1441/2007. The temperature range at which these products are normally transported and stored in the market is 4-8°C. The purpose of the current work was to establish whether these products can be classified as RTE foods unable to support the growth of *L. monocytogenes* at 4°C and to determine whether the pathogen has the ability to proliferate in the products under conditions of temperature abuse (12 and 22°C). For each of the 27 different experimental conditions (product, inoculum level and storage temperature) the fate of the pathogen was monitored in duplicate for a period up to 12 months according to the ISO 11290-1 and -2 protocols, resulting in a total of 54 growth/survival curves. The behaviour of the pathogen in the PCAs was product- and storage temperature-dependent. Hence, whereas none of the three products supported growth of *L. monocytogenes* at 4°C, one product supported growth at 12°C and all products supported *L. monocytogenes* growth at 22°C. In the 4°C trials as well as in the 12°C-trials of two out of the three PCAs, the *L. monocytogenes* populations declined continuously, but slowly over time. As a result, in the high and medium inoculum trials, viable *L. monocytogenes* could be detected even after 12 months of storage. The results showed that the products tested do not support the growth of *L. monocytogenes* under proper refrigeration (4°C). However, every effort should be exercised to prevent post-pasteurization product contamination and product temperature abuse, because a hypothetical contamination with *L. monocytogenes* can persist in the products for a considerable time under refrigeration or can lead to pathogen outgrowth under conditions of severe temperature abuse.

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