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Heat resistance of *Clostridium sordellii* spores

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ABSTRACT

The thermal destruction kinetics of *Clostridium sordellii* spores was studied in this research. Decimal reduction times (*D* values) for *C. sordellii* ATCC 9714 spores ranged between 175.60 min for D_{80} (the *D* value for spore suspensions treated at 80 °C) and 11.22 min for D_{95}. The thermal resistance (*Z*) and temperature coefficient (Q_{10}) values of spores were calculated to be as high as 12.59 °C and 6.23, respectively. At 95 °C, the relative thermal death rate and relative thermal death time of *C. sordellii* ATCC 9714 spores were found to be 0.0085/min and 118 min, respectively, indicating that the death rate of spores was 118 times lower at 95 °C than at 121.1 °C. Heat treatments at up to 85 °C for 120 min failed to cause a 100-fold destruction in spore populations of *C. sordellii* ATCC 9714. By contrast, spore counts were reduced by $2\log_{10}$ cycles within 73 min and 23 min at 90 °C and 95 °C, respectively. This is the first published report of thermal inactivation of *C. sordellii* spores; however, further studies are needed to confirm these results in real food samples.

1. Introduction

Clostridium sordellii is an anaerobic, Gram-positive, spore-forming rod with peritrichous flagella. It is widely distributed in nature and is commonly found in the soil and in the intestines of animals, including 0.5% of all humans [1,2]. The organism is an uncommon human pathogen, although it has been more thoroughly described in veterinary medicine [3,4]. Many of the strains are non-pathogenic, however, virulent strains cause lethal infections in animal species, such as enteritis and enterotoxaemia in sheep and cattle and myonecrosis and gangrene in humans. Virulence is attributed to various exotoxins, even though only two, the lethal and hemorrhagic toxins, have been extensively studied [1].

Turcsán [5] isolated clostridia spores from goose livers, at rates up to $2.0\log_{10}$ CFU/g, during commercial processing procedures. The isolates were subsequently identified biochemically as *C. sordellii* using the API rapid ID 32 A test strip (bioMérieux, Marcy-l'Etoile, France). Hungary is among the world's top producers of raw goose liver. This product is regarded as a Hungarian specialty in many countries. Large quantities of this commodity are exported to France. In fact, Hungary's goose liver exports make up approximately two-thirds of goose liver imports by France. The further-processed product, known as foie gras, is a great delicacy [6,7]. Although the foodborne pathogenicity of *C. sordellii* is uncertain [8], the presence of *C. sordellii* spores in goose liver products may pose a health risk to consumers of these commodities.

It is necessary to use reduced temperatures during commercial processing of raw goose livers because French products made from less contaminated raw materials are heat treated at temperatures ranging from 95 °C to 98 °C, whereas Hungarian semi-preserved livers are normally heated at 105–108 °C, thereby causing significant deterioration in sensory properties of the final products. For this reason, the primary purpose of the present work was to determine heat treatment parameters, i.e., temperature and holding time combinations below 100 °C, which result in a $2\text{-}\log_{10}$ reduction in *C. sordellii* spore counts. To our knowledge, no study on thermal inactivation of *C. sordellii* spores has been published previously.

2. Materials and methods

2.1. Microorganism

C. sordellii ATCC 9714 was obtained in freeze-dried form from the Pasteur Institute (Paris, France). It was incubated at 37 °C for 7 days in Reinforced Clostridial Medium (RCM; Merck KGaA, Darmstadt, Germany), and then a concentrated suspension was produced by centrifugation in sterile 30-mL tubes using a Sigma 3K12 centrifuge (Sigma Laborzentrifugen GmbH, Osterode, Germany) at 4500*g* for 15 min at 10 °C. The supernatant was removed, and a pure suspension was prepared by dilution with quarter-strength Ringer's solution.

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2.2. Enumeration of vegetative cells and spores

After purification, vegetative cell counts and initial spore counts were determined. For the determination of vegetative cell counts, unheated samples were pour-plated on Plate Count (PC) agar (Merck) and Tryptose Sulfite Cycloserine (TSC) agar (Merck). Initial spore counts were enumerated in samples heated at 80 °C for 10 min and then plated on PC and TSC agars (Merck). The plates were incubated at 37 °C for 72 h. Anaerobic conditions were generated using anaerobic culture jars (2.5 L) and AnaeroGen AN 25 sachets (Oxoid Ltd, Basingstoke, UK).

2.3. Induction of sporulation

In order to promote sporulation of *C. sordellii* ATCC 9714, the content of each 30-mL centrifuge tube (i.e., the concentrated cell suspension referred to in Subsection 2.1.) was poured into 500-mL aliquots of a special sporulation broth developed by Schaeffer et al. [9]. In our previous trials, this culture medium proved to be superior to that proposed by Duncan and Strong [10] to induce sporulation of *Clostridium perfringens*. The samples were incubated anaerobically at 37 °C for 72 h and were subsequently stored refrigerated at 4 °C for 24 h.

2.4. Thermal inactivation

For heat resistance determinations, each of 10 mL of *C. sordellii* ATCC 9714 spore suspensions were placed in a GFL 1003 temperature controlled water bath (Gesellschaft für Labortechnik mbH, Burgwedel, Germany) set at 80 °C, 85 °C, 90 °C or 95 °C and kept for 120 min, 120 min, 96 min, and 36 min, respectively. Samples were removed at 5 to 9 predetermined time intervals, depending on the inactivation temperature. After removal, the samples were immediately immersed into an ice-water-bath at 2 °C, and the spore counts of heat treated suspensions were determined by the pour-plate technique using PC agar (Merck) incubated at 37 °C for 48 h under anaerobic conditions as described above. Colony forming units (CFU) were recorded and converted into $\log_{10}$ CFU/mL.

2.5. Calculation of heat resistance values

Each experiment was performed in duplicate and repeated three times. The data were presented as means and standard deviations. Survival curves were plotted to determine decimal reduction times (D value). D values were obtained by taking the reciprocal of the slope from linear regression of the survival curves. The Z value (the temperature increase required for a 10-fold reduction of D value) was estimated by plotting the $\log_{10}$ D values against heating temperatures and taking the reciprocal of the slope from linear regression. The temperature coefficient (Q_{10} value) was determined on the basis of the Z value according to the following formula:

$$Q_{10} = 10^{\frac{10}{Z}}.$$

Relative thermal death rates (RTDR) and relative thermal death times (RTDT) were also calculated from the Z value and heating temperatures (T) as follows:

$$RTDR = \frac{1}{RTDT} = 10^{\frac{T-121.1\ ^\circ C}{Z}}.$$

Table 1
Decimal reduction time (D), $\log_{10}$ D and $\log_{10}$ 12D values[a] of *Clostridium sordellii* ATCC 9714 spores.

Temperature (°C)	D value (min)	$\log_{10}$ D value	$\log_{10}$ 12D value
80	175.60 ± 15.68	2.24 ± 0.04	3.32 ± 0.04
85	90.55 ± 6.83	1.96 ± 0.03	3.04 ± 0.03
90	36.24 ± 1.89	1.56 ± 0.02	2.64 ± 0.02
95	11.22 ± 0.14	1.05 ± 0.01	2.13 ± 0.01

[a] Values are means ± standard deviations, based on six observations (two samples, three replicates).

3. Results and discussion

The initial viable counts of *C. sordellii* ATCC 9714 vegetative cells ranged from 6.8×10^3 CFU/mL to 7.7×10^3 CFU/mL, whereas no *C. sordellii* ATCC 9714 spores ($<1.0 \times 10^0$ CFU/mL) were found in the same suspensions after heating at 80 °C for 10 min. Anaerobic incubation at 37 °C for 72 h of *C. sordellii* ATCC 9714 suspensions in the sporulation broth [9] resulted in final spore counts above 5.0$\log_{10}$ CFU/mL.

As shown in Fig. 1, the thermal survivor curves for *C. sordellii* ATCC 9714 spores followed first-order kinetics, suggesting that as the spore population was heated at a specific temperature, the spores died at a constant rate [11]. The R^2 values of the survival curves were ≥0.97. As expected, the treatment temperature of 80 °C resulted in limited inactivation on *C. sordellii* ATCC 9714 spores, however, spore counts were substantially reduced, i.e., from 2.7×10^5 CFU/mL to 1.3×10^4 CFU/mL, when heated at 85 °C for 120 min. At 90 °C, a decrease of over 2$\log_{10}$ cycles was observed in *C. sordellii* ATCC 9714 spore counts, as the initial value of 2.4×10^5 CFU/mL declined to 5.2×10^2 CFU/mL over the 96-min thermal treatment period. By heating the test suspensions at 95 °C

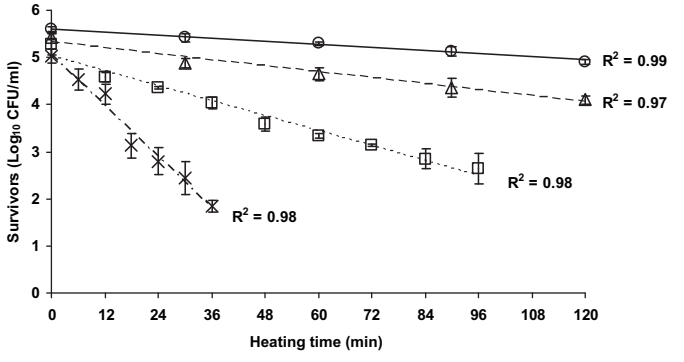

Fig. 1. Survival curves of *Clostridium sordellii* ATCC 9714 spores at 80 °C (○), 85 °C (△), 90 °C (□), and 95 °C (×). Whiskers indicate standard deviations calculated from six observations (two samples, three replicates).

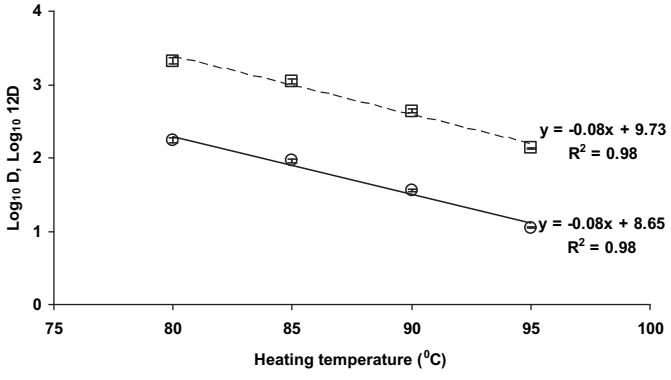

Fig. 2. Thermal death time [$\log_{10}$ D (○) and $\log_{10}$ 12D (□)] curves for *Clostridium sordellii* ATCC 9714 spores over the temperature range of 80–95 °C. Whiskers indicate standard deviations calculated from six observations (two samples, three replicates).

Table 2
Relative thermal death rates and times of *Clostridium sordellii* ATCC 9714 spores.

Temperature (°C)	Relative thermal death rate (RTDR) (1/min)	Relative thermal death time (RTDT) (min)
80	0.0005	1839
85	0.0014	737
90	0.0034	295
95	0.0085	118

for 36 min, a 3-$\log_{10}$ reduction (from 1.2×10^5 CFU/mL to 7.1×10^1 CFU/mL) of *C. sordellii* ATCC 9714 spores was achieved.

The decimal reduction times for *C. sordellii* ATCC 9714 spores in this research ranged between 175.60 min for D_{80} (the *D* value for spore suspensions treated at 80 °C) and 11.22 min for D_{95} (Table 1). Because, to the best of our knowledge, there are no other reported *D* values for *C. sordellii* spores, the data obtained in this study could only be compared with those in the published literature on the heat resistance of other clostridial spores.

Bradshaw et al. [12] reported *D* values of 12.48–22.42 min at 98.9 °C for *C. perfringens* spores suspended in phosphate buffer. In a study by Heredia et al. [13], when heat resistance of *C. perfringens* spores was determined in double-distilled water, the *D* values at 85 °C and 95 °C of 55 min and 24 min, respectively, were reported. Byrne et al. [14] determined that thermal destruction times for *C. perfringens* spores in pork luncheon roll were 30.6 min (D_{90}), 9.7 min (D_{95}) and 1.9 min (D_{100}). Sarker et al. [15] provided evidence suggesting that spores of *C. perfringens* food-poisoning isolates carrying a chromosomal *C. perfringens* enterotoxin (*cpe*) gene are 60-fold more heat resistant than spores of *C. perfringens* isolates carrying a plasmid *cpe* gene are. The D_{100} values for spores produced by the chromosomal and plasmid *cpe* isolates ranged from 30 min to 124 min and 0.5–1.9 min, respectively [15]. By contrast, Juneja et al. [16] reported D_{100} values of 15.5–21.4 min in beef gravy for spores of the *C. perfringens* strains surveyed by Sarker et al. [15].

Differences in *D* values obtained in various studies may be attributed to several factors. Sporulation media and temperature used for spore preparation, recovery conditions, presence of inhibitory substances, temperature and time of incubation and, most importantly, the presence or absence of lysozyme in the recovery media affect the calculated spore heat resistance [16]. Scott and Bernard [17] suggested that there may be significant variations among strains and among the reported *D* values by different investigators within the same strains.

After logarithmic transformation, decimal reduction times (*D* and 12*D*) were plotted versus heating temperature. Thermal death time curves were thus obtained, and *Z* and Q_{10} values were then calculated from the slope of the curves (Fig. 2). The *Z* value of *C. sordellii* ATCC 9714 spores was found to be 12.59 °C, indicating that an increase of 12.59 °C was required to change the *D* value to transverse by 1$\log_{10}$. Mah et al. [18] reported a somewhat lower *Z* value of 10.76 °C for *Clostridium sporogenes* PA 3679 spores in a sporulation medium supplemented with either $CaCl_2$ or $CaCO_3$ at a final concentration of 0.5%, and an even lower *Z* value (8.3 °C) was reported by Byrne et al. [14] for *C. perfringens* spores in pork luncheon roll. In this study, the Q_{10} value of *C. sordellii* ATCC 9714 spores was calculated to be as high as 6.23, indicating that an increase of 10 °C in treatment temperature resulted in a thermal death rate 6.23 times higher than the initial one.

The calculated *Z* value was used to determine the relative thermal death rate (RTDR) and relative thermal death time (RTDT) of *C. sordellii* ATCC 9714 spores at the specific temperatures applied in this work. The obtained RTDR and RTDT values are presented in Table 2. At 95 °C, the RTDR and RTDT values of *C. sordellii* ATCC 9714 spores were 0.0085/min and 118 min, respectively, indicating that the death rate of spores was 118 times lower at 95 °C than at 121.1 °C.

In conclusion, heat treatments at up to 85 °C for 120 min failed to ensure the required degree of destruction in spore populations of *C. sordellii* ATCC 9714. In contrast, spore counts were reduced by 2$\log_{10}$ cycles within 73 min and 23 min at 90 °C and 95 °C, respectively. Further studies are needed to confirm these results in real food matrices. However, the findings of this study may be beneficial to the poultry processing industry in designing thermal pasteurization processes to effectively eliminate *C. sordellii* spores in semi-preserved goose liver products. The data may also be used in future risk assessment studies.

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