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International Journal of Food Microbiology 80 (2002) 261–264

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Short communication

Barotolerance of *Staphylococcus aureus* is increased by incubation at below 0 °C prior to hydrostatic pressure treatment

Seiji Noma*, Isao Hayakawa

Laboratory of Food Process Engineering, Division of Food Biotechnology, Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

Received 11 June 2001; received in revised form 18 November 2001; accepted 21 March 2002

Abstract

The effect of preincubation under low temperatures on inactivation of *Staphylococcus aureus* IFO 13276 by hydrostatic pressure treatment (HPT) was investigated. Preincubation before HPT was carried out by submerging cell suspension in an ethylene glycol bath at temperatures from 30 to −20 °C for 15 min. After HPT at the same temperatures, survivors of incubated *S. aureus* was not significantly ($P>0.05$) influenced when preincubation took place at temperatures above 0 °C. Survivors of incubated *S. aureus*, however, were approximately two log cycles higher when preincubation took place at temperatures below 0 °C. This increase in barotolerance of *S. aureus* was not observed in the presence of 40 μg/ml of chloramphenicol.

Keywords: *Staphylococcus aureus*; Adaptation; Low temperature; Barotolerance

1. Introduction

Hydrostatic pressure treatment (HPT) is an emerging technology in food preservation (Reyns et al., 2000). Previous researchers have shown that HPT at low temperatures, especially below 0 °C, is effective in inactivating microorganisms such as *Escherichia coli*, *Streptococcus lactis* and *Saccharomyces cerevisiae* (Hayakawa and Furukawa, 2000; Takahashi et al., 1991; Sonoike et al., 1993). *Staphylococcus aureus*, however, appeared to be resistant to HPT at low temperatures (Gervilla et al., 1999, 2000; Takahashi et al., 1991). Gervilla et al. (1999) described that no significant difference was observed in barotolerance of *S. aureus* between 25 and 0 °C. Takahashi et al. (1991) reported the barotolerance of *S. aureus* at −20 °C to be similar to the tolerance at 20 °C. The barotolerance of *S. aureus* could represent a significant problem for the practical application of pressure technology in food preservation, and clarification of the possible causes of high barotolerance is important.

Both Gervilla et al. (1999) and Takahashi et al. (1991) subjected *S. aureus* cell suspension to incubation before HPT for adjusting its temperature for the HPT. It is well known that microorganisms can accommodate a variety of changing conditions and stresses in their environment in order to survive and multiply (Berry and Foegeding, 1997; Giard et al., 1996). It is also known that a microbial response to a

* Corresponding author. Tel.: +81-92-642-3018; fax: +81-92-642-3015.

E-mail address: nomas@agr.kyushu-u.ac.jp (S. Noma).

0168-1605/02/$ - see front matter
PII: S0168-1605(02)00142-3

particular stress can lead to a higher resistance against the other stresses (Jenkins et al., 1988, 1990; Pichereau et al., 2000). Therefore, it was hypothesized that high resistance of *S. aureus* to HPT under low temperatures could be obtained by an adaptation to low temperature during incubation prior to HPT.

In this study, effects of preincubations prior to HPT at low temperatures on the barotolerance of *S. aureus* IFO 13276 were investigated.

2. Materials and methods

2.1. Bacterium

S. aureus IFO 13276 was obtained from the Institute for Fermentation Osaka (Osaka, Japan).

2.2. Preparation of cell suspension

S. aureus was cultivated in 10 ml of nutrient broth (Eiken Chemical, Japan) at 30 °C for 15 h. Cells were harvested and washed three times by centrifugation at $7000 \times g$, 30 °C for 15 min, and were then resuspended in 0.9% (w/v) sodium chloride solution with and without chloramphenicol added to a final concentration of 40 μg/ml at 30 °C to give approximately 2×10^6 CFU/ml of final density.

2.3. Preincubation conditions

Cell suspension sealed in a 1.5-ml sterile screw-capped plastic sample tube (Greiner Labortechnik, Kremsmünster, Germany) was preincubated in an ethylene glycol-thermocontrolled bath (Haake, GH, Germany) at 30, 5, 0, −5, −10 and −20 °C for 15 min prior to hydrostatic pressure treatment. Time required for the temperature downshift from 30 to −5 °C and from 30 to −20 °C were 2 and 5 min, respectively. These periods were excluded from the preincubation time. Unincubated (normal) cells of *S. aureus* were defined as *S. aureus* cells kept at 30 °C until pressure treatments.

2.4. Hydrostatic pressure treatment (HPT)

Suspensions of *S. aureus* were subjected to HPT at 200 MPa for 60 min. The prototype high-pressure apparatus (Yamamoto Suiatsu Kogyosho, Osaka, Japan) used for this treatment was described in a previous paper (Hayakawa et al., 1994). The time needed to reach 200 MPa was approximately 15 s, and the pressure release time was approximately 0.1 s. Treatment temperatures were the same as the preincubation temperature, e.g. cells preincubated at −5 °C were pressurized at −5 °C and cells preincubated at 0 °C were pressurized at 0 °C, and that were regulated by a thermocontroller NCB-2400 (Tokyo Rikakikai, Tokyo, Japan) using ethylene glycol as a refrigerant.

2.5. Measurement of surviving cells

After HPT, appropriate serial dilutions of cell suspension were prepared with physiological saline and 0.1 ml volume was plated onto nutrient agar (Eiken Chemical, Tokyo Japan). The number of surviving cells was enumerated as colony forming units (CFU)/ml after incubation at 30 °C for 48 h.

2.6. Statistical analysis

All experiments were done in triplicate. The data presented was the means of each experiment. Significant differences of barotolerance were determined with 5% level of significance ($P < 0.05$) by Student's *t* test.

3. Results and discussion

Survivors of *S. aureus* after HPT are shown in Fig. 1. At HPT temperatures from 30 to 0 °C, *S. aureus* followed the general inactivation behavior at decreasing temperatures (Sonoike et al., 1993), and no significant difference ($P > 0.05$) was observed in survivors between preincubated and normal cells of *S. aureus*. At temperatures from −5 to −20 °C, normal cells of *S. aureus* showed the same inactivation pattern. However, preincubated *S. aureus* did not follow the same pattern of inactivation behavior, and their barotolerance corresponded to that at 30 °C, i.e. it did not decrease with decreasing HPT temperatures. These results suggested that the barotolerance of *S. aureus* was increased through adaptation to low temperature during preincubation. In the presence of chloramphenicol (Fig. 2),

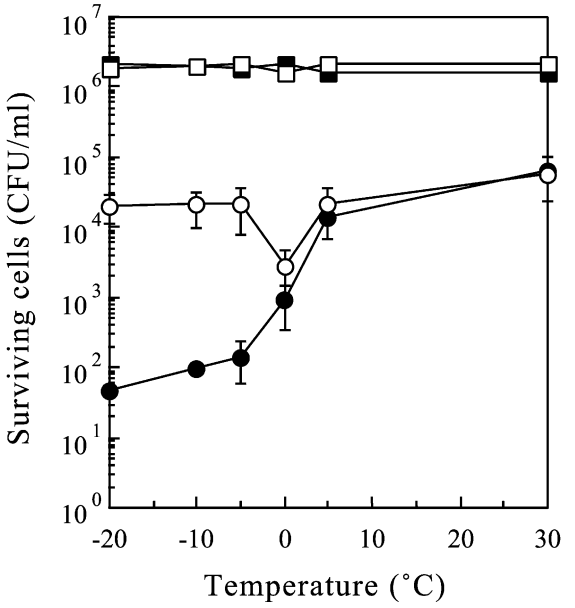

Fig. 1. Survivors of *S. aureus* after HPT at 200 MPa for 60 min. Circular and square symbols indicate the survivors after HPT at 200 and 0.1 MPa, respectively. Open and close symbols show preincubated and normal *S. aureus*, respectively.

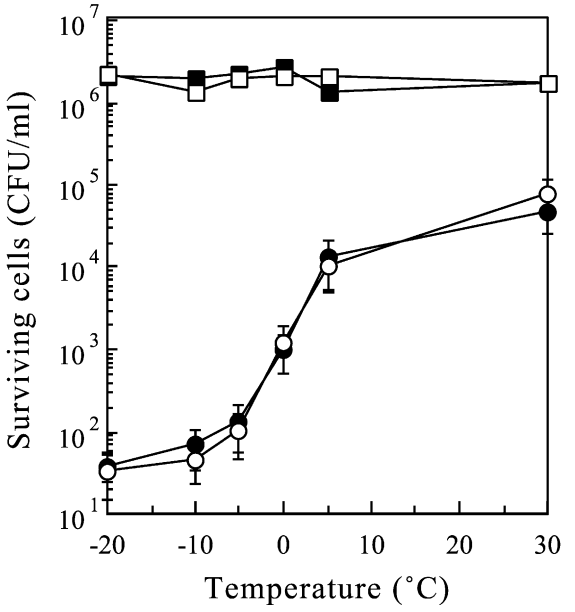

Fig. 2. Survivors of *S. aureus* after HPT at 200 MPa for 60 min under presence of chloramphenicol. Circular and square symbols indicate the survivors after HPT at 200 and 0.1 MPa, respectively. Open and close symbols show preincubated and normal *S. aureus*, respectively.

no significant differences ($P>0.05$) were observed in the barotolerance between preincubated and normal cells of *S. aureus* for all temperatures tested. These results suggest that the barotolerance of cells under conditions of inhibition of protein synthesis could not be increased by preincubation at below 0 °C.

In conclusion, it was considered that the increase in barotolerance of *S. aureus* at below 0 °C resulted from adaptation to low temperature during preincubation. This increase is important from the practical standpoint because it could lead insufficient inactivation. Therefore, it is necessary to carefully examine the accurate barotolerance of *S. aureus*.

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