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# Cold Adaptation of *Lactobacillus paraplantarum* C7 Isolated from Kimchi

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**Abstract** The effect of preadaptation at low temperature on cryoprotection was studied for *Lactobacillus paraplantarum* C7, a bacteriocin producer isolated from kimchi. *L. paraplantarum* C7 cells in their log growth phase were incubated at 15°C, 10°C, or 5°C for 2, 4, and 6 h, respectively, before being frozen at -70°C. After 24 h of freezing, viable cells were counted after brief thawing. The freezing-thawing cycles were repeated three more times. Cells preadapted at 10°C or 5°C before freezing survived better than control cells, but preadaptation at 15°C did not confer cryoprotection. Chloramphenicol addition did not destroy the cryoprotection, indicating that protein synthesis was not required for the development of cryoprotection. SDS-PAGE showed induction of a 6.5-kDa protein, a major cold-shock protein, in preadapted cells.

**Key words:** Kimchi, cold adaptation, *Lactobacillus paraplantarum*, cold-shock protein

All organisms, including bacteria, produce a group of proteins when encountering suboptimally low temperatures. Proteins induced by low temperatures are called cold-shock proteins or cold-inducible proteins, and they are collectively believed to protect cells from adverse effects due to low temperatures [9, 10]. Cold-shock proteins and their corresponding genes have been characterized from *E. coli* [15], *Bacillus subtilis* [2], *Lactococcus lactis* [11, 12], and other Gram-positive bacteria. The most abundant cold-shock proteins are a family of closely related low molecular weight proteins, about 6.5–7 kDa in size, and they share a high degree of sequence identity (≥45%) [10]. Kimchi fermentation occurs at low temperatures, usually less than 10°C, since kimchi ripened at low temperatures has better organoleptic quality than kimchi prepared at room temperature (RT) [7, 8]. Therefore, it is reasonable to expect that lactic acid bacteria (LAB) in kimchi constantly face a low temperature challenge and must have developed a mechanism(s), which protects them and enables them to maintain viability at a refrigerating temperature. So far, however, no researches have been conducted on the cold-shock response of LAB originated from kimchi. Understanding of the cold-shock response of kimchi LAB is important not only for basic researches on LAB, but also for production of high-quality kimchi, because successful growth of LAB at low temperatures is a prerequisite for successful kimchi ripening. Thus, knowledge on the cold-shock response of kimchi LAB will contribute to the development of strains better suited for kimchi production. In this work, induction of cryoprotection in *L. paraplantarum* C7 was confirmed by comparing the survival ratios of cells preadapted at low temperatures with cells without preadaptation after freezing-thawing challenge. The presence of a 6.5 kDa major cold-shock protein in the extracts from preadapted *L. paraplantarum* C7 cells was also confirmed by SDS-PAGE.

## Bacterial Strain and Growth Condition

*Lactobacillus paraplantarum* C7 was isolated from kimchi, and regular cabbage kimchi was purchased from a local supermarket. Biochemical properties and 16S rDNA sequencing data were used to identify C7, as described by Kwon *et al.* [5]. Detailed identification procedure together with other properties of *L. paraplantarum* C7 will be described elsewhere. *L. paraplantarum* C7 was grown at 25°C on MRS broth (Difco Lab, Detroit, U.S.A.) or on MRS plate solidified with 1.5% agar.

## Cold Adaptation of *L. paraplantarum* C7 Cells

*L. paraplantarum* C7 was cultured in 5 ml of MRS broth overnight, and 200 ml of fresh MRS broth was inoculated with the overnight culture (1%) and incubated at 25°C.

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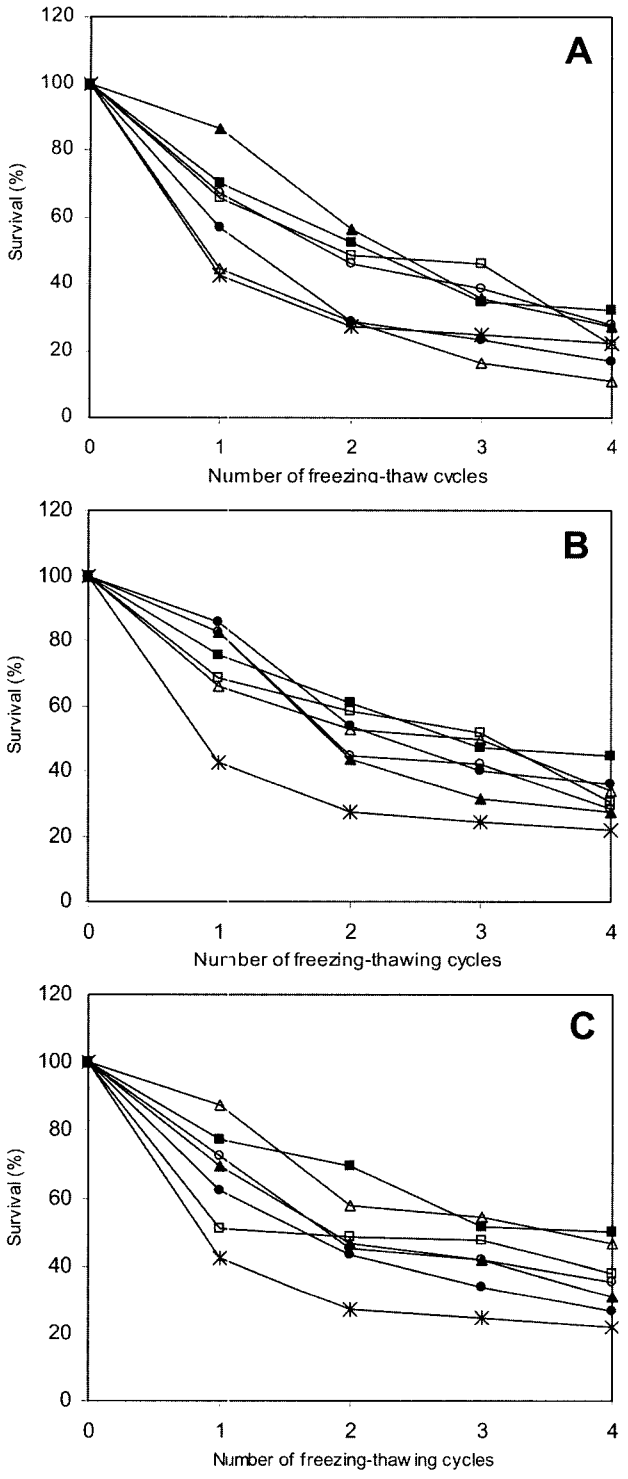


**Fig. 1.** Survival ratios of *L. paraplantarum* C7 cells after freezing-thawing cycles.
Survival ratio was calculated as the percentage of survived cells after each freezing-thawing cycle relative to the cell number before freezing (100%). Cm (chloramphenicol) was added at a concentration of 100 μg/ml. (A), Cells preadapted at 15°C; (B), cells preadapted at 10°C; (C), cells preadapted at 5°C. Control cells without preadaptation (*), cells exposed to a low temperature for 2 h (○), 4 h (□), or 6 h (△), and cells preadapted in the presence of Cm for 2 h (●), 4 h (■), or 6 h (▲).

When the $OD_{600}$ reached 0.6, aliquots of 9 ml were dispensed into 19 tubes. Cold adaptation was carried out by placing cells into low temperature circulating water baths where temperature had been adjusted to 5°C, 10°C, or 15°C, and adaptation was continued for 2, 4, and 6 h, respectively, and then cells were quickly frozen and stored at -70°C. Control cells were frozen without preadaptation. After 24 h of freezing, cells were thawed by placing tubes in a 25°C water bath for 5 min. Aliquots were taken out for viable cell counting and cells were then frozen again at a -70°C freezer. Total four cycles of freezing-thawing were repeated. Thawed cells were serially diluted with 1% peptone water, and viable cells were counted by spreading 100 μl of diluted samples on MRS plates. Three plates were used for each appropriately diluted sample, and cell numbers were averaged. Survival ratio was calculated by dividing viable counts obtained after each freezing-thawing cycle with the viable counts before the first freezing. The results are shown in Fig. 1. Twenty-two percent of control (w/o preadaptation) cells survived after four cycles of freezing-thawing. Survival ratios of cells preadapted at 5°C were 35.4, 37.8, and 46.8% for 2, 4, and 6 h adaptation after four cycles, respectively. Survival ratios of cells preadapted at 10°C were 28.3, 30.3, and 34.1% for 2, 4, and 6 h adaptation, respectively. The results indicated that preadaptation at low temperatures enhanced the viability of *L. paraplantarum* C7 cells upon freezing, but the degree of increase was much smaller than those reported for other LAB (see below). Preadaptation at 15°C, however, did not enhance cryoprotection. These results suggest that, upon exposure to low temperatures, some changes occurred in *L. paraplantarum* C7 cells, eventually resulting in increase of cryoprotection. Although *L. paraplantarum* C7 is a mesophile, similar to most of the LAB isolated from kimchi (see Fig. 2), C7 seems to have developed ways to allow

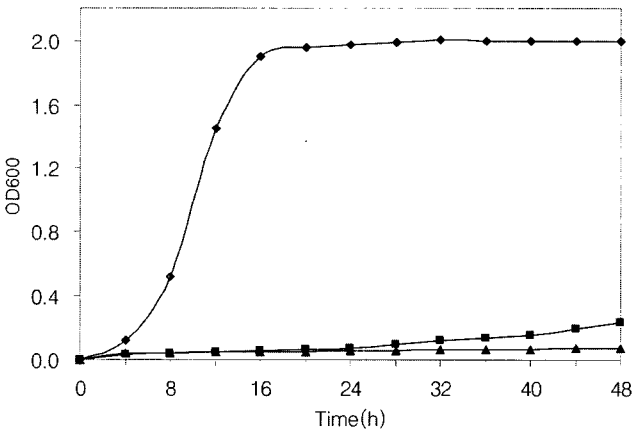


**Fig. 2.** Growth of *L. paraplantarum* C7 at different temperatures.
Overnight culture of *L. paraplantarum* C7 (at 25°C) was inoculated at 1% into fresh MRS broth and incubated at different temperatures. -◆-, 25°C; -■-, 10°C; -▲-, 5°C.

high cell viability at low temperatures which are encountered during kimchi ripening and storage. That might be at least part of the reasons why preadaptation at 15°C was not effective for developing cryoprotection: In other words, the temperature of 15°C might not be low enough to trigger any protective response(s) for *L. paraplantarum* C7. This interpretation would also explain the result that preadaptation at 5°C gave the best cryoprotection. In this regard, the cold-shock response of *L. paraplantarum* C7 appears to be quite different from that of *Streptococcus thermophilus* CNRZ302, a thermophile, which showed less than 0.01% survival ratio after 4 cycles of freezing-thawing, but the survival ratio increased by 100- 1,000-fold after preadaptation at 20°C for 2 or 4 h [13]. Such large differences between *S. thermophilus* and *L. paraplantarum* indicate again the fact that C7 might have been well adapted to low temperature environments, thus maintaining higher basal level of resistance against freezing, whereas *S. thermophilus*, a thermophile, depends heavily on preadaptation for cryoprotection.

Contrary to our expectation, chloramphenicol addition did not significantly reduce the viability of preadapted cells upon freezing. Since chloramphenicol is a known inhibitor for protein and DNA synthesis, the result indicates that *de novo* protein synthesis is not required for the development of cryoprotection. The presence of chloramphenicol during preadaptation decreased the viability of *Streptococcus thermophilus* CNRZ 302 [13] and *Lactococcus lactis* MG1363 [12] to the level of unadapted control cells. For *L. paraplantarum* C7, preadaptation for 2 or 4 h at 10°C in the presence of chloramphenicol (100 μg/ml) even slightly increased the viability. A possible explanation is that synthesis of some cold-labile proteins was prevented by chloramphenicol, which contributed to the improved viability of cells at low temperatures. A similar observation of chloramphenicol addition was reported for the acid-stress response of *Lactococcus lactis* sub. *lactis* [1].

### SDS-PAGE

Protein extracts from *L. paraplantarum* C7 cells were prepared as described by Kim *et al.* [3] with some modifications. Cells grown overnight (10 ml) were harvested by centrifugation and resuspended in 1 ml of 50 mM phosphate buffer, pH 7.0, and 0.4 g of sterile glass beads (Sigma, 106 μm) were added. Cells were vortexed at highest speed for 30 s, followed by cooling on ice for 30 s. Total five cycles of vortexing-cooling were repeated. Disrupted cells were centrifuged, and supernatant was obtained as the protein extract. SDS-PAGE of the protein extracts was performed according to the method of Laemmli [6], using the SE 400 Sturdier Electrophoresis unit (Hoefer Pharmacia Biotech. Inc.) and a 12% acrylamide gel. After electrophoresis, gel was stained with Coomassie brilliant blue G-250, and the result is shown in Fig. 3. Overall protein patterns between

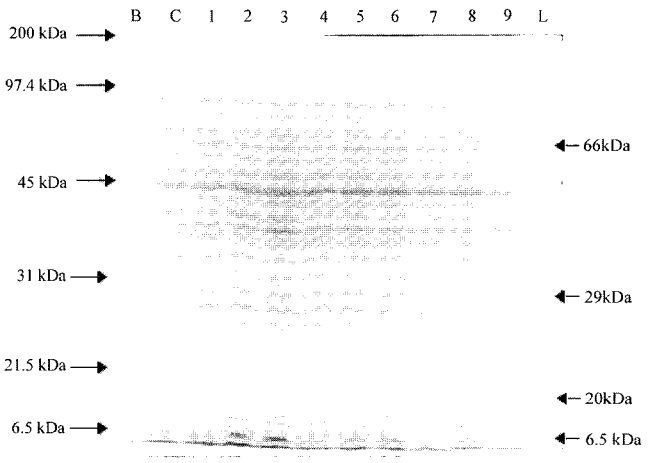


**Fig. 3.** SDS-PAGE of protein extracts from *L. paraplantarum* C7 cells adapted at low temperature for different periods of time. B, Broad range size marker (BioRad); L, low range size marker (Sigma); C, control cells (no adaptation); 1, cells adapted at 15°C for 2 h; 2, cells adapted at 15°C for 4 h; 3, cells adapted at 15°C for 6 h; 4, cells adapted at 10°C for 2 h; 5, cells adapted at 10°C for 4 h; 6, cells adapted at 10°C for 6 h; 7, cells adapted at 5°C for 2 h; 8, cells adapted at 5°C for 4 h; 9, cells adapted at 5°C for 6 h.

control and preadapted cells were quite similar, except for a 6.5 kDa protein which was apparently induced in the preadapted cells. The 6.5 kDa band was apparent in the cells preadapted at 15°C or 10°C for 4 or 6 h, but not apparent in the cells preadapted at 5°C. The size of the protein band agreed well with the size of the known major cold-shock proteins, and also with the report in which only a 6.5 kDa band was different between control and preadapted cells [4]. It is not clear why the 6.5 kDa cold-shock protein was not produced in cells adapted at 5°C, at which temperature *L. paraplantarum* C7 showed the best cryoprotection. It is quite possible that the 6.5 kDa cold-shock protein may indirectly enhance the survival of *L. paraplantarum* C7 after freezing by inducing other proteins (cold-inducible proteins) which provide cryoprotection [14], but SDS-PAGE may not be sensitive enough to detect its synthesis. In summary, this report demonstrated a cold-shock response of *L. paraplantarum* C7, which was isolated from kimchi. Similar to other LAB from kimchi, C7 has a high basal level of cold resistance, therefore, the cold-shock response of kimchi LAB seems to be different from mesophilic or thermophilic LAB.

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## REFERENCES


1. Axel, H., B. Sandrine, J. C. Girard, A. Benachour, P. Boutibonnes, and Y. Auffray. 1996. The lactic acid stress response of *Lactococcus lactis* susp. *lactis*. *Curr. Microbiol.* **33:** 194-199.
2. Graumann, P., T. M. Wendrich, M. H. W. Weber, K. Schroder, and M. A. Marahiel. 1997. A family of cold shock proteins in *Bacillus subtilis* is essential for cellular growth and for effcient protein synthesis at optimal and low temperatures. *Mol. Microbiol.* **25:** 741-756.
3. Kim, T.-W., S.-H. Jung, J.-Y. Lee, S.-K. Choi, S.-H. Park, J.-S. Jo, and H.-Y. Kim. 2003. Identification of lactic acid bacteria in kimchi using SDS-PAGE profiles of whole cell proteins. *J. Microbiol. Biotechnol.* **13:** 119-124.
4. Kim, W. S., N. Khunajakr, and N. W. Dunn. 1998. Effect of cold shock on protein synthesis and on cryotolerance of cells frozen for long periods in *Lactococcus lactis*. *Cryobiology* **37:** 86-91.
5. Kwon, D. Y., M. Koo, C. R. Ryoo, C.-H. Kang, K.-H. Min, and W. J. Kim. 2002. Bacteriocin produced by *Pediococcus* sp. in kimchi and its characteristics. *J. Microbiol. Biotechnol.* **12:** 96-105.
6. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680-685.
7. Mheen, T. I. and T. W. Kwon. 1984. Effect of temperature and salt concentration on kimchi fermentation. *Korean J. Food Sci.Technol.* **16:** 443-450.
8. Rhie, S. G. and S. K. Chun. 1982. The influence of temperature on fermentation of kimchi. *Korean J. Nutri. Food* **11:** 63-66.
9. Thieringer, H., A. P. G. Jones, and M. Inouye. 1998. Cold shock and adaptation. *BioEssays* **20:** 49-57.
10. van de Guchte, M., P. Serror, C. Chervaux, T. Smokvina, S. D. Ehrlich, and E. Maguin. 2002. Stress responses in lactic acid bacteria. *Antonie van Leeuwenhoek* **82:** 187-216.
11. Wouters, J. A., F. M. Rombouts, W. M. de Vos, O. P. Kuipers, and T. Abee. 1998. Clustered organization and transcriptional analysis of a family of five *csp* genes of *Lactococcus lactis* MG1363. *Microbiology* **144:** 2885-2893.
12. Wouters, J. A., B. Jeynov, F. M. Rombouts, W. M. de Vos, O. P. Kuipers, and T. Abee. 1999. Analysis of the role of 7 kDa cold-shock proteins of *Lactococcus lactis* MG1363 in cryoprotection. *Microbiology* **145:** 3185-3194.
13. Wouters, J. A., F. M. Rombouts, W. M. de Vos, O. P. Kuipers, and T. Abee. 1999. Cold shock proteins and low-temperature response of *Streptococcus thermophilus* CRZ302. *Appl. Environ. Microbiol.* **65:** 4436-4442.
14. Wouters, J. A., M. Mailhes, F. M. Rombouts, W. M. de Vos, O. P. Kuipers, and T. Abee. 2000. Physiological and regulatory effects of controlled overproduction of five cold shock proteins of *Lactococcus lactis* MG1363. *Appl. Environ. Microbiol.* **66:** 3756-3763.
15. Yamanka, K., L. Fang, and M. Inouye. 1998. The CspA family in *Escherichia coli*: Multiple gene duplication for stress adaptation. *Mol. Microbiol.* **27:** 247-255.