CROSS PROTECTION BY HEAT AND COLD SHOCK TO LETHAL TEMPERATURES IN *CLOSTRIDIUM PERFRINGENS*

Santos García; Julio C. Limón; Norma L. Heredia*

Laboratorio de Bioquímica y Genética de Microorganismos, Departamento de Microbiología.
Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León. San Nicolás, Mexico

ABSTRACT

A heat shock pre-treatment of *Clostridium perfringens* provides protection against lethality from subsequent exposure to cold shock and vice versa. Heat shocked cells were more cold tolerant (one log reduction in 65 min) than control cells (one log reduction in 34 min). On the other hand, cold-shocked cells were more thermotolerant (D_{55}= 17 min) than the control (D_{55}= 6.5 min). The addition of chloramphenicol (an inhibitor of protein synthesis) in the experiments indicates that synthesis of new proteins was necessary for cross protection between both treatments.

Key words: *Clostridium perfringens*, heat shock, cold shock

INTRODUCTION

Several authors have reported that when organisms are exposed to high sublethal temperatures, they are able to survive higher lethal temperatures (3,15,19). On the other hand, sublethal cold shock enables cells to survive to lower lethal temperatures (1,5,11,12).

C. perfringens is responsible for one of the most common types of human food-borne illness. This is a gram-positive, spore forming, rod-shaped, encapsulated and non motile bacterium. The generally accepted temperature range for growth is between 15 and 50ºC (14). The most important characteristic of *C. perfringens* relative to food safety is its ability to grow rapidly at elevated temperatures (4). The potential impact of such an ability on food safety is obvious. In our laboratory, we have observed that when cells were exposed to heat shock, they acquired heat tolerance (7).

Recently, several reports have indicated that cross protection between different lethal or adverse treatments can be induced in some microorganisms (9,10,21,22). However, no information is available about protection between heat and cold treatments. Because of the exposure of several foods to low and high temperatures during its preparation and storage, it is important to study the ability of the food-borne pathogen *C. perfringens* to survive extreme temperatures. In this work, we examined the effect of sublethal high and low temperatures on the cold and heat tolerance, respectively, of this bacterium. Furthermore, the role of new protein synthesis on tolerance was determined by the use of chloramphenicol (an inhibitor of protein synthesis) in the experiments.

MATERIALS AND METHODS

An enterotoxin-positive strain, FD-1041 of *C. perfringens* was used. It was kindly provided by S. Harmon from the Food and Drug Administration, Washington, D.C. The strain was maintained as a stock spore culture in Cooked Meat medium (Difco, Detroit, MI) at -20ºC. Active cultures were obtained by transferring two drops of the stock culture into screw capped tubes containing 10 ml of Fluid Thioglycolate Medium (FTG, Difco), heat-activating (75ºC / 15 min) and incubating overnight (16-18 h) at 37ºC (6).

From an activated culture, 13 x 100 mm tubes containing two ml of fresh FTG were inoculated (1% inoculum) and incubated at 43ºC. Caution was taken to inoculate directly on the medium, and avoid contaminating the top internal surface of the tube. When

* Corresponding Author. Mailing address: Laboratorio de Bioquímica y Genética de Microorganismos, Departamento de Microbiología. Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León. Apto. Postal 124-F. San Nicolás, Mexico. Telefax: (+52-8) 3763044. E-mail: nheredia@ccr.dsi.uanl.mx.

cells reached mid-log phase ($A_{600} = 0.6$-0.7), the culture was treated one of two ways. To determine the induction of cold tolerance as a result of a previous heat shock, some cultures were immersed in a water bath at 50ºC for 30 min. At the beginning of this treatment, chloramphenicol (200 µg/ml final concentration) was added to some cultures (18). Control cultures were not heated. Following the 30 min, all the cultures were immediately submerged in a refrigerated water bath at 10ºC for one h. Viable cells were enumerated by plate count at 20 min intervals using a medium composed of 1% yeast extract, 1.5% Bacto Tryptone and 1.5% agar (Difco). Pour plates were incubated anaerobically in a mixture of CO_2 and N_2 (90:5) at 37ºC. Colonies were counted after 48 h and the data plotted. Death curves of the different treatments were obtained by the least-squares regression method. The time required to reduce the number of viable cells by one log ("D" value) was determined from the slope of the linear portion of the death curves (4).

To determine induction of heat tolerance as a result of a previous cold shock, several tubes were immersed in the refrigerated water bath at 15°C, for 30 min. Chloramphenicol was added to several cultures as described before. Control tubes were not cold shocked. Following the 30 min treatment, all tubes were immediately submerged in a hot water bath at 55°C for 15 min. Viable cells were determined at five min intervals as previously described. "D" values were determined.

Due to innate resistance of *C. perfringens* at 55 or 10°C, analyses of tolerance at these temperatures were carried out at intervals of 5 or 20 min respectively. In this case, cells were damaged more rapidly at the higher temperature than the lower. All experiments were done at least in triplicate. The "t" method was used to determine differences between slopes from death curves (23).

RESULTS AND DISCUSSION

Our results indicate that a cross protection between both treatments is produced in *C. perfringens* as a result of temperature stresses. Cells submitted previously to cold shock (15°C for 30 min) were significantly ($P < 0.05$) more resistant to 55°C when compared to the control (Fig. 1). D values for cold-treated cells were 17 min as compared to 6.5 min for control cells (Table 1). These results indicated that cold shocked cells were 2.6 times more heat tolerant than the control. No significant difference in heat tolerance was observed between control cells and those receiving chloramphenicol.

Cells previously submitted to a heat shock induced by increasing the temperature from 43 to 50°C, were almost two fold more resistant ($P < 0.05$) to cold temperature (10°C) than those of the control (Fig. 2). In this case, the time required to reduce the population one-log was 65 min for heat-treated cells, while the same reduction was achieved in 34 min in the control (Table 1). Also, no significant difference in cold tolerance was observed

between control cells that were treated or untreated with chloramphenicol and shocked cells treated with chloramphenicol. Viable cell counts of cultures treated with chloramphenicol increased after cells were transferred to a fresh antibiotic-free medium (data not shown).

Some authors have reported that exposure to a stress condition can enable microorganisms to become resistant to other kinds of

Table 1. Decimal reduction times at high and low temperatures of previously cold and heat shocked cells respectively of *C. perfringens*.

Treatment	D value (min) ($P = 0.05$)	
	55ºC	10ºC
Control	6.5 ± 0.5	34 ± 4
Control + Chloramphenicol	5.2 ± 0.2	37 ± 3.5
Shocked cells*	17 ± 1^A	65 ± 2.5
Shocked cells + Chloramphenicol	6.1 ± 0.3^A	33 ± 2

*: cells were previously shocked at (15°C for 30 min)[A] or (50°C for 30 min).
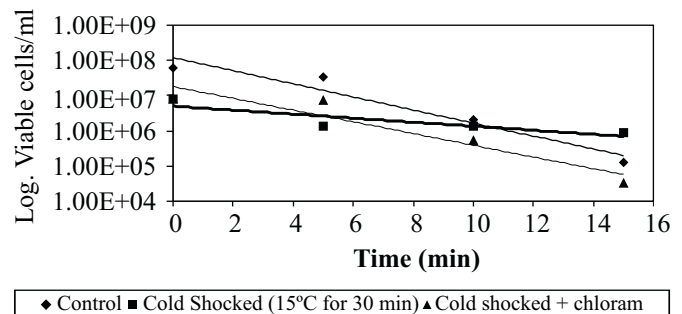

Figure 1. Inactivation of *C. perfringens* cells at 55°C. Cells were previously cold shocked and in some cases chloramphenicol was added.
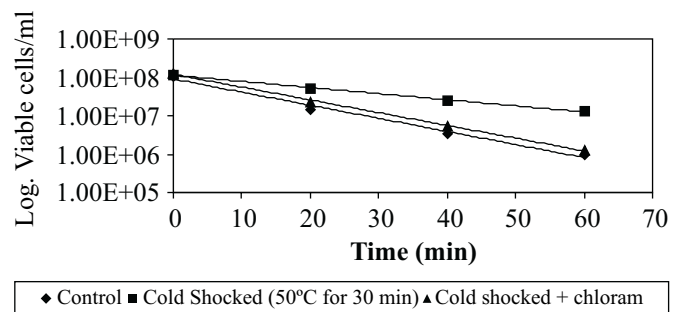

Figure 2. Inactivation of *C. perfringens* cells at 10°C. Cells were previously heat shocked nd in some cases chloramphenicol was added.

stresses. In some microorganisms, heat shock induces protection against subsequent exposure to salt (21) and hydrostatic pressure (11) stresses, and increases superoxide dismutase activity (2). On the other hand, cold shock induces protection against acrylic acid and copper ions (22). Although in *Escherichia coli*, a strong cold shock represses synthesis of heat shock proteins (11), in other microorganisms several proteins are induced by both heat and cold shock (8,13,16,20). Recently it was reported that *E. coli* exposed to heat, cold, oxidative and tetracycline-induced stresses secreted exometabolites that protected the cells against the same or other stresses (17). The present study demonstrated that heat shock induced protection against cold shock and vice versa in *C. perfringens*. The presence of chloramphenicol (an inhibitor of protein synthesis) in the experiments indicates that synthesis of new proteins during the first shock is necessary for cross protection between both treatments. Studies are under way to determine the proteins involved in both stresses in *C. perfringens* and to determine if this cross protection is also exhibited by *C. perfringens* in foods and contribute to foodborne disease outbreaks.

Therefore, protection against lethal hot or cold temperatures was achieved by *C. perfringens* as a result of a previous exposure to the counterpart sublethal temperature. The effect of temperature tolerance on the biomedical aspects of food safety may be important, because of the widespread application of thermal processing or refrigeration storage for the control of pathogenic microorganisms associated with food.

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RESUMO

Proteção cruzada por choque pelo calor e frio à temperaturas letais em *Clostridium perfringens*

Um pré-tratamento com choque térmico em *Clostridium perfringens* resulta em proteção contra letalidade a uma posterior exposição a choque pelo frio, e vice-versa. Células submetidas a choque pelo calor foram mais tolerantes ao frio (redução de um log em 65 min) do que células controle (redução de um log em 34 min). Por outro lado, células submetidas à choque pelo frio foram mais termotolerantes (D_{55}=17 min) do que os controles (D_{55}=6,5 min). A adição de cloranfenicol (um inibidor de síntese protéica) nos experimentos indica que síntese de novas proteínas era necessária para a proteção cruzada pelos dois tratamentos.

Palavras-chave: *Clostridium perfringens*, choque térmico, choque pelo frio

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