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To cite this article: L S Chernova *et al* 2020 *J. Phys.: Conf. Ser.* **1695** 012049

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Overexpression of the recombinant IbpA protein from *Acholeplasma laidlawii* in *Escherichia coli* cells increases thermotolerance

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Abstract. The presence of a gene encoding small heat shock protein IbpA in *Acholeplasma laidlawii* (*A/IbpA*) appears to be one of the key factors determining the high adaptive capabilities of this mycoplasma. Previously, we showed a participation of the N- and C-terminal regions of *A/IbpA* in functions of chaperone *in vitro*. The aim of this work was to establish the involvement of the N- and C-terminal motifs of the recombinant *A/IbpA* in the survival of *Escherichia coli* cells under temperature stress *in vivo*. To determine this, we used genetically engineered versions of the *A/IbpA* with truncations and mutations in the N- and C-terminal domains. Viability was determined by counting CFU and differential fluorescent staining. Studies have shown that hyperproduction of the N-terminal-modified IbpA from *A/IbpA* is able to exhibit thermotolerance *E. coli* cells.

This research was funded by the Russian Science Foundation (project No. 17-74-20065), the microscopy assays were funded by RFBR (project № 20-34-90066).

1. Introduction

Small heat shock proteins of α -crystalline type (sHSPs) play a key role in the cell survival under stress conditions. The primary function of sHSPs is preventing the irreversible denaturation and aggregation of partially denatured proteins [1, 2]. Some sHSPs are known to interact with cytoskeletal proteins and to protect them from denaturation under stress conditions [3; 4]. They are also necessary for the cell membrane fluidity regulation, since they stabilize the liquid-crystal state of the bilayer and maintain the membrane integrity during thermal fluctuations [5; 6]. Their importance for the cell survival under extremely unfavorable conditions may also be confirmed by their presence in most well studied microorganisms that can exist in dormant state forms. Remarkably, sHSPs were not found in many bacteria which belong to Mollicutes (mycoplasmas). Among them sHSP homologs were identified only in some members of the Spiroplasmataceae and Anaeroplasmataceae families, and to a greater extent in bacteria of Acholeplasmataceae family, including *Acholeplasma laidlawii* [7]. In contrast to most mycoplasmas which are parasites of human and animals, *A. laidlawii* is the only mollicute that is able to exist free of any host [8] and survives under a variety of stresses. We suggest that IbpA protein (the sHSP orthologue) can be one of the key factors governing the extensive stress adaptive abilities of *A. laidlawii*. Our early investigations suggest that IbpA participates both in the stabilization of individual polypeptides and probably in the maintenance of various cellular structures upon temperature stress [9]. Recently we have shown that IbpA protein with truncated or mutated N-terminal domain forms fibrils and exhibits higher chaperon activity *in vitro* [1].

In this paper we report that overexpression of the recombinant IbpA with truncated or mutated N-terminal domain increases the viability of *E. coli* cells under heat shock.

2. Materials and Methods

2.1 Bacterial strains and growth conditions

Escherichia coli BL21 was used in this assay. Plasmids providing the overexpression of recombinant mutated and truncated IbpA proteins from *A. laidlawii* used in this study were obtained earlier by cloning



of the truncated *ibpA* genes into pET15b vector [1,7]. Bacteria were grown in LB broth with rigorous shaking at 37°C for 24 hours and subjected to heating for 1 h at various temperatures as indicated.

2.2 Evaluation of survival of IbpA overproducing *E. coli* cells under heat shock

E. coli BL21 pIbpA and *E. coli* BL21 pET15b cells were grown in LB medium until OD₆₀₀~0.6 and IPTG was added until the final concentration of 1 mM followed by cultivation during 1 h. Next the cells were exposed to the heat shock (56°C) for 1 h. Cells from the same aliquots before and after the heat treatment were seeded on a solid LB medium and CFUs were calculated after 24h cultivation at 37°C. Alternatively, cells subjected to the heat shock for 1 h were stained by acridine orange and propidium iodide and analyzed by differential fluorescent microscopy.

2.3 Statistical analysis

Experiments were carried out in three biological repeats. The fraction of non-viable cells in microscopic images was estimated as the relative fraction of the red cells among all cells in the combined images obtained by overlaying of the green and the red fluorescence microphotographs (10 images per each sample) by using BioFilmAnalyzer software [10].

3. Results

One of the known functions of sHSPs is the enhancement of cellular resistance to heat shock by prevention of partially denatured proteins aggregation. As shown previously, sHSP from the rice increased the tolerance of *E. coli* cells to the 30 min exposure to 47.5 °C [11]. In our recently research *AIbpA* with damaged N-terminal domain was characterized by increased chaperone activity of sHSP *in vitro* [1], so we asked whether an overexpression of modified *AIbpA* would affect the survival of the recombinant *E. coli* cells under heat shock conditions. *E. coli* BL21 cells carrying either pIbpA plasmids providing the expression of IbpA proteins with various mutations/truncations of N- and C-terminal domains or pET15b vector (the latter used as control) were induced by the addition of IPTG followed by 1 h incubation at 30 °C for protein overproduction. Next the cells were incubated at 56 °C for 1 h, and CFUs were calculated before and after the exposure to heat shock. Count of viable cells with empty pET15b vector decreased approximately 10-fold after heat treatment. In contrast, the cells producing recombinant IbpA with truncated (N12, ΔN25) or mutated (ΔN11N12) N-terminus survived after heating suggesting that IbpA increases the cell survival under the heat shock (Figure 1 A).

Additionally to the CFUs counting, the viability of the cells after heat shock was evaluated by differential fluorescent microscopy (see figure 1 B-L). Cells were stained by both acridine orange and propidium iodide and the fraction of non-viable cells was estimated as the relative fraction of the red cells (Figure C-L). Similarly to CFUs count data, an increased viability of cells producing IbpA with truncated (N12, ΔN25) or mutated (ΔN11N12) N-terminus has been observed.

Taken together our results indicate that the overexpression of N-termini-modified IbpA from *AIbpA* more effectively protects *E. coli* cells from heat shock than other variants including full-sized *AIbpA* itself.

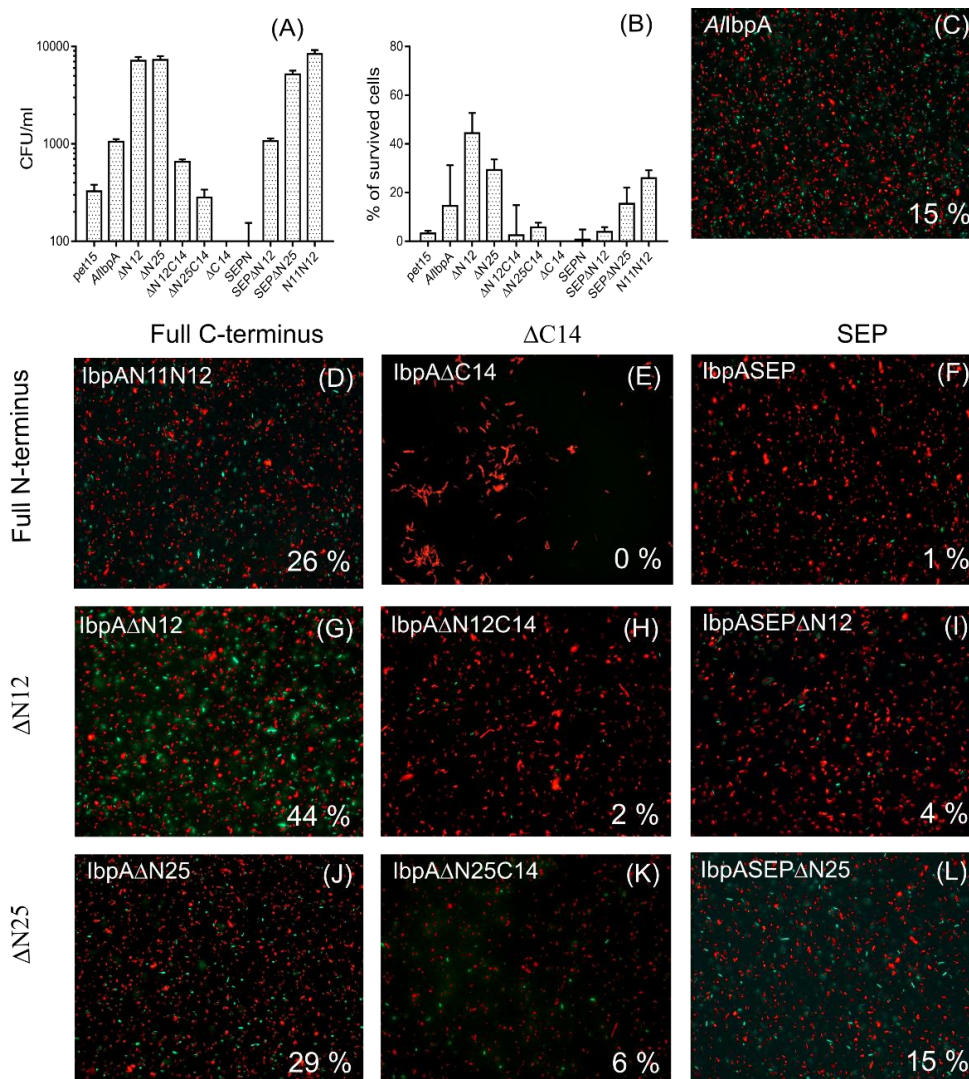


Figure 1 Viability of the cells after heat shock **(A)** CFUs counting; **(B- L)** differential fluorescent microscopy. The removed parts from either N- or C-termini are shown in Figure 1: $\Delta N12$ lacks the first 12 amino acid residues; $\Delta N25$ lacks the first 25 amino acid residues; in N11N12 both F11F12 are substituted by N11N12; from the C-terminus were removed 14 amino acids by obtaining $\Delta C14$; in SEPN L134 and L136 replaced by S134 and P136; $\Delta N12C14$, $\Delta N25C14$, $SEP\Delta N12$ and $SEP\Delta N25$ with truncated both N- and C-termini.

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