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Assaying the Presence of Histone-Like Protein HU in *Halobacillus karajensis*

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Abstract: Histone-Like Proteins (HLPs) in bacteria are small basic proteins that contribute to the control of gene expression, recombination, DNA replication and compressing the bacterial DNA in the nucleoid. Among the HLPs, HU protein as a dimer plays an important role in binding to DNA and bending it. In this study, we showed that a 9.5-10 kDa protein with the same electrophoretic mobility as HU exists in *Halobacillus karajensis* which is a novel gram positive moderate halophile bacterium that was recently isolated from surface saline soil of the Karaj Region, Iran. The genes encoding HU protein were also assayed during this study by Polymerase Chain Reaction.

Key words: DNA binding proteins, histone-like proteins, HU, *Halobacillus karajensis*

INTRODUCTION

All higher organisms contain small, basic, abundant, deoxyribonucleic acid (DNA)-binding proteins called histones (McGhee and Felsenfeld, 1980). Bacteria contain proteins that share some properties like amino acid sequence, electrostatic charge, low molecular weight and binding to DNA with eukaryotic histones and therefore are called Histone-Like Proteins (HLPs) (Drlica and Rouviere-Yaniv, 1987). These proteins play important roles in compacting DNA, initiation of replication, transcription, repair and regulation of gene expression (McGhee and Felsenfeld, 1980; Talukder *et al.*, 1999; Arthanari *et al.*, 2004; Saiz and Vilar, 2007). Among the proteins associated with the DNA HU, IHF, H-NS, Fis and StpA have been mainly studied (Falconi *et al.*, 1996; Kostrewa *et al.*, 1991; Laurent winter *et al.*, 1997; Tendeng and Bertin, 2003).

The *Escherichia coli* HU protein was initially discovered and purified by Rouviere-Yaniv and Gros (1975) as a small (9 kDa), basic (pI 8.57) heterodimer which is a thermo stable factor associated with the *Escherichia coli* nucleoid and stimulates *in vitro* RNA synthesis. It participates in important cellular functions such as initiation of replication, transcription and site-specific recombination (Kamau *et al.*, 2005). The genes coding this protein are *hup* α and *hup* β . It has 90 amino acid

residues in each monomer (McGhee and Felsenfeld, 1980) and resembles H1 and H2B in its amino acids sequence (Drlica and Rouviere-Yaniv, 1987).

HU belongs to a family of proteins, which is classified into three subgroups (Sundiatu *et al.*, 1999; Pontiggia *et al.*, 1993; Goodrich *et al.*, 1990; Greene *et al.*, 1986).

The *Bacillus subtilis* protein HBSu has been crystallized and analysis of X-ray diffraction patterns leads to a model in which two identical monomers interlock. In fact, the two HU subunits form a body with two α -helix arms that wrap around the minor groove of DNA (McGhee and Felsenfeld, 1980; Kamau *et al.*, 2005). This protein does not have any specific binding site and prefers DNA aberrations such as four way junctions and cruciform structures (Aitken and Rouviere-Yaniv, 1979; Kamau *et al.*, 2005; Zhang *et al.*, 2006).

In this study we assayed the presence of some histone-like proteins specially HU in *Halobacillus karajensis* which is a gram positive, novel moderate halophile bacterium, recently isolated from surface saline soils of Karaj region in Iran (Amoozegar *et al.*, 2003). Since, the histone-like proteins have not been studied in any of the genus *Halobacillus*, we found it interesting to study them specially HU, which is one of the main HLPs as a vital protein in *H. karajensis* a gram-positive bacterium.

MATERIALS AND METHODS

Bacterial Strain, growth media, growth curve: *Bacillus subtilis* grown in LB (NaCl 1%, tryptone 1%, yeast extract 0.5 g) at 33°C. *Halobacillus karajensis* strain Ma-2¹ grown in NB 10% (10% NaCl) and NA 10% (NaCl 10%), pH 7-7.2 at 34°C.

The growth curve was drawn in 24 h for *H. karajensis* at 620 nm.

Preparation of DNA extracts and PCR: DNA extraction was performed for *H. karajensis* and *B. subtilis* by a kit purchased from Sigma. PCR was performed according to Sinagen protocol with a gradient concentration of 0.5 to 1.5 mM from MgCl₂ and a temperature range of 44-52°C.

Detection of HU coding Gene in *Halobacillus karajensis* and *Bacillus subtilis*: The degenerate primers used for amplification of internal sequences of HU coding genes were FHV and RHV (Table 1). They were designed using *hbsu* gene in *B. subtilis*. These primers amplify a 240 bp fragment between nucleotides 1 and 240 on the *hbsu* gene of *Bacillus subtilis*.

Preparation of protein extracts

Johns method: Partially purified HU extracts were prepared as follows: A 700 mL culture of the *H. karajensis* was grown in NB 10% in shaker incubator for 4-6 h at 33°C. The biomass was collected after 4-6 h of incubation by 20 min centrifugation at 12000 rpm and resuspended in 3-5 volumes of cold PCA 5% and vortexed for 5 min. The suspension was sonicated at 180 W, 9 kHz at 4°C for 15 min. Then stirred for 30 min and spun for 2 min in 12000 rpm and the supernatant was collected in a clean micro tube. The remaining precipitant was resuspended in 3-5 volumes of cold PCA 5%, vortexed for 5 min, stirred for 30 min and spun for 2 min at 12000 rpm. The resulting supernatant was collected in a micro tube and the same process was done once again on the precipitant. All subsequent steps were performed in 4°C. HCl 6 N was added to the supernatant slowly until 0.3 N final normality was reached and then 3-3.5 volumes of acetone was added and spun for 5-10 min at 1500 rpm at 15-18°C. Then three volumes of 6:1 ratio of (Acetone: HCl 0.1 N) was added to the precipitant and spun for 5-10 min at 1500 rpm at 15-18°C. The same procedure was repeated for the resulting precipitant. The final protein extract was washed with three volumes of Acetone and frozen in -70°C for further use (Johns, 1964).

Ammonium Sulfate precipitation (65-90%):

H. karajensis cells growing in NB containing 10% NaCl were harvested 4 h after inoculation and suspended in buffer A (20 mM Tris-HCl (pH 7.8), 20 mM NH₄Cl, 10 mM

Table 1: The sequences of used primers

Temperature (°C)	Primer sequence	Primer name
48.1	5'ATGAACAARACAGAWMTRAT3'	FHV
47.6	5'YTTRAAWGCWGGNDYTTT 3'	RHV

(CH₃COO)₂Mg, 5 mM 2-mercaptoethanol) at 4 volumes g⁻¹ of wet weight. The suspension was disrupted by sonic oscillation (3 min, 180 watts, 9 kHz) at 4°C. The sonicate was centrifuged at 15,000 g for 20 min at 4°C, followed by 260,000 g for 2 h. Supernatants were fractionated with ammonium sulfate (65-90%) (Nakamura *et al.*, 1999).

The final extract was loaded on SDS PAGE 15% and stained by Coomassie blue (Laemmli, 1970).

RESULTS AND DISCUSSION

Considering the fact that the gene or genes encoding HU protein has not yet been defined and sequenced in *Halobacillaceae*, we chose *Bacillus subtilis* as the positive control in this research. *Bacillus subtilis* belongs to the *Bacillaceae* family, which is phylogenetically close to *Halobacillaceae* and the HU protein is widely studied in it.

In order to assay the presence of the gene encoding HU in *H. karajensis*, DNA extraction was carried out according to Sigma Kit on both *B. subtilis* and *H. karajensis*. As shown in Fig. 1, the DNA extracts were loaded on agarose 1% gel.

The DNA extracts were then amplified by primers FHV and RHV, which were designed according to the

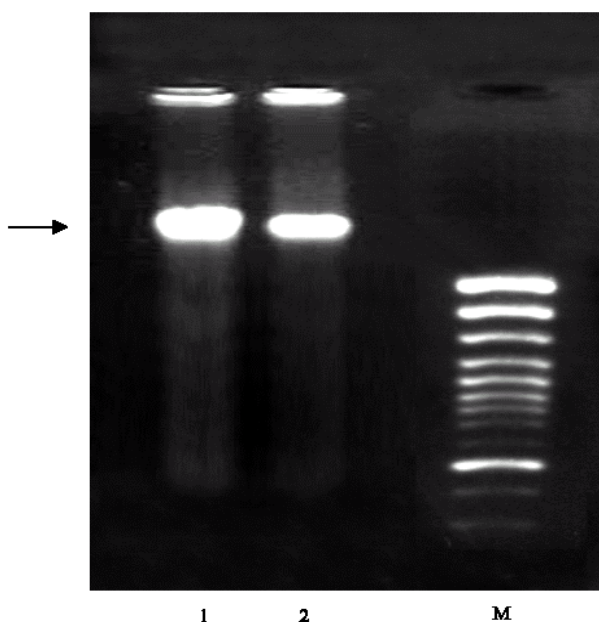


Fig. 1: 1-DNA extracts from *Bacillus subtilis* by Sigma Kit, 2-DNA extracts from *H. karajensis* by Sigma Kit, M-DNA marker

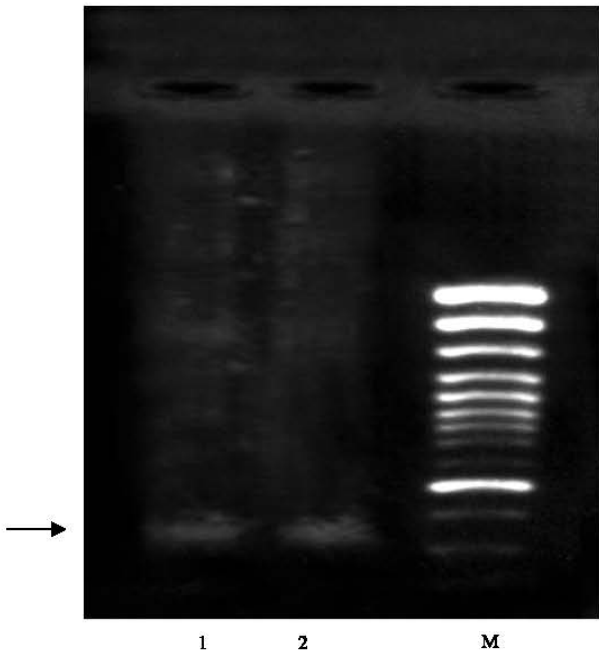


Fig. 2: 1 and 2-240 bp PCR product for HU coding gene in *B. subtilis* by FHV and RHV primers M-DNA marker

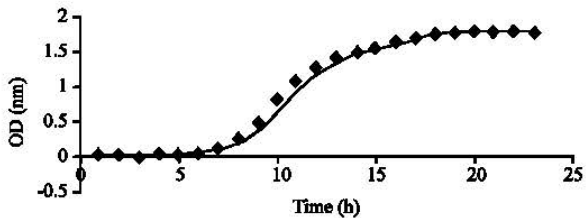


Fig. 3: *H. karajensis* growth curve

consensus sequence of *hup* genes in different *Bacillaceae*. A 240 bp PCR product was obtained from *B. subtilis* but no results were seen for *H. karajensis* probably because the 3' OH of the designed primers did not anneal correctly with the HU gene in *H. karajensis* (Fig. 2).

As mentioned before, since HU family proteins are 20% identical in their amino acid sequence (Drlica and Rouviere-Yaniv, 1987) and none of the *Halobacillaceae* genus was sequenced, the nearest genus to these bacteria that is *Bacillaceae* was used for designing the degenerate primers. Therefore, primers that are specific for *hup* of *B. subtilis* might not be specific enough for amplifying the HU coding gene in *H. karajensis*.

While assaying the genes encoding HU protein in *H. karajensis* we encountered some problems, so we carried on with looking for the HU protein in these bacteria.

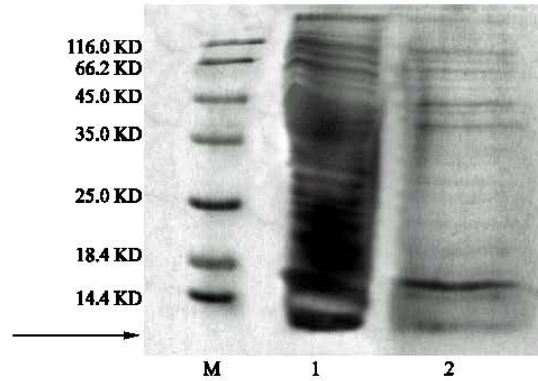


Fig. 4: Protein Extracts from *H. karajensis* obtained by ammonium sulfate precipitation 75% (1) and 80% (2) are loaded near marker, which is shown by M. The protein with the same electrophoretic pattern as HU is shown with an arrow

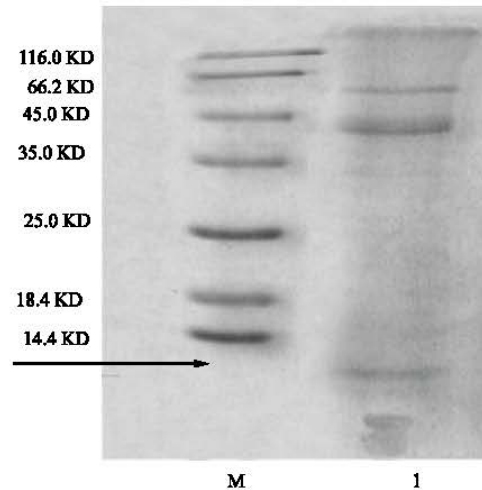


Fig. 5: Protein extracts from *H. karajensis* by Johns' method (1) are loaded near marker, which is shown by M. The protein with the same electrophoretic pattern as HU is shown with an arrow

Before preparing protein extracts from *H. karajensis* the growth curve was drawn in 24 h at 620 nm. The protein extraction was carried out during the exponential phase where HU is most abundant (Fig. 3).

Protein extracts by Ammonium sulfate precipitation (75 and 80%) and Johns method were loaded on SDS PAGE 15% and stained with coomassie blue (Fig. 4 and 5). The electrophoretic mobility was similar to that of HU in gram-positive bacteria and the presence of a 9.5 kDa protein was estimated by drawing the protein standard curve for the gel (Fig. 6).

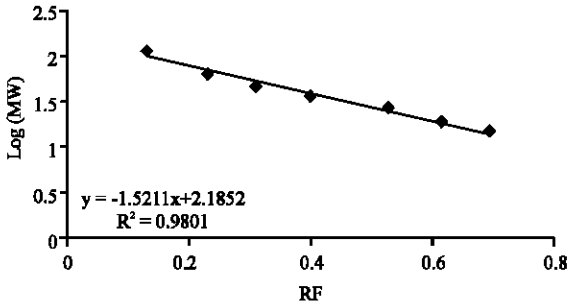


Fig. 6: The standard curve drawn using the molecular weight log of the marker proteins

Since HU and H1 have identical amino acid sequences and biochemical properties (Drlica and Rouviere-Yaniv, 1987), we used this eukaryotic histone for assaying whether immunological properties of these two proteins are similar.

Immunochemical properties of the protein with the same molecular weight as HU that is similar to H1 in amino acid contents were investigated by western blotting using anti H1 antibody (Ghadam and Rabbani, 2002). Observing no results was because of the difference between the antigenic properties of the two proteins H1 and HU (data not shown).

CONCLUSION

This study showed that a protein with the same molecular weight of *Bacillus subtilis* HU exists in *H. karajensis* and the gene sequence of the protein has some difference from *Bacillus subtilis* and determination of the gene encoding the protein needs more investigations.

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