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A comparative study of ribosomal and DNA binding protein II from two thermophilic bacteria, *Bacillus caldolyticus* strain EP 00275 and *Bacillus stearothermophilus***

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Ribosomal and DNA binding proteins (DNA bp II) from an extreme thermophilic bacterium, *B. caldolyticus* strain EP 00275, were investigated for stability and crystallization and compared to the homologous proteins from *B. stearothermophilus*. Two-dimensional gel electrophoresis of both types of proteins, the amino acid composition and the sequences of some of the peptides of DNA bp II revealed a close relationship between each other. The physico-chemical characteristics of DNA bp II were similar but different from homologous proteins from *T. thermophilus* and *C. pasteurineum*. From our results we conclude: *B. stearothermophilus* and *B. caldolyticus* strain EP 00275 are similar organisms with regard to their ribosomal and DNA binding proteins.

Thermophilic bacteria; Ribosomal protein; DNA binding protein II; Crystallization

1. INTRODUCTION

Thermophilic bacteria have found widespread use as a source of thermostable proteins which are highly suitable tools, i.e. for polymerase chain reaction method in order to amplify selective discrete segments of DNA [1]. Knowledge of the three-dimensional structure of these proteins provides more information on the precise mechanism of the reaction per se.

The isolation and characterization of three extreme thermophilic *Bacilli* have been described [2]. One of these species, namely *B. caldolyticus* with an optimal growth temperature of 72°C, appears to be suitable for structural studies on proteins. It is commercially available as *B. caldolyticus* strain EP 00275. In a comparative study we have looked at: (i) the yield of ribosomes, (ii) the two-dimensional gel pattern (2D) of the ribosomal proteins, (iii) the amino acid composition of tryptic peptides and the sequence of some of these peptides of DNA binding protein II (DNA bp II), (iv) the stability, crystallization and binding of DNA bp II to DNA, rRNA, ribosomes and ribosomal subunits. The results show that by these criteria *B. caldolyticus* strain EP 00275 is essentially indistinguishable from *B. stearothermophilus*.

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2. MATERIALS AND METHODS

Frozen *B. caldolyticus* cells were purchased from CAMR, Porton (UK). The cells had been grown in batch culture at 65°C (strain number EP 00275).

Ribosomes and DNA pb II were prepared by gel filtration [3,4] and subunits were separated by zonal centrifugation [5]. Ribosomal proteins were extracted [6] and separated by 2D [7]. The DNA bp II was characterized by amino acid sequence studies [8], urea and temperature-denaturing experiments and NMR [9], respectively. The binding DNA properties of DNA bp II from *B. caldolyticus* were studied by filter-binding assay [10] as well as by gel filtration of the nucleic acid-protein and of the ribosome-protein complexes [4]. The electron microscopy of nucleic acid-protein complexes was performed [11] using plasmid DNA RSF 1010 (gift from Dr E. Lanka, Berlin). The DNA bp II from *B. caldolyticus* was crystallized by the hanging drop vapor diffusion method [13].

3. RESULTS

From 2 kg of *B. caldolyticus* cells 140000 A_{260} units (8.754 g) of 70 S ribosomes were obtained. The 2D patterns of the 30 S and 50 S proteins from the two *Bacillus* species are shown in Fig. 1. The distribution and the number of protein spots are very similar in the two species. Small differences in the positions of a few of the proteins can be observed. The yield of DNA bp II was 80 mg from 2 kg cell paste. On 2D gels the protein occupied a position characteristic of DNA bp II, just to the left of L23. In Fig. 2 the protein is shown with a background of 50 S ribosomal proteins. The co-electrophoresis experiments show that the DNA bp II

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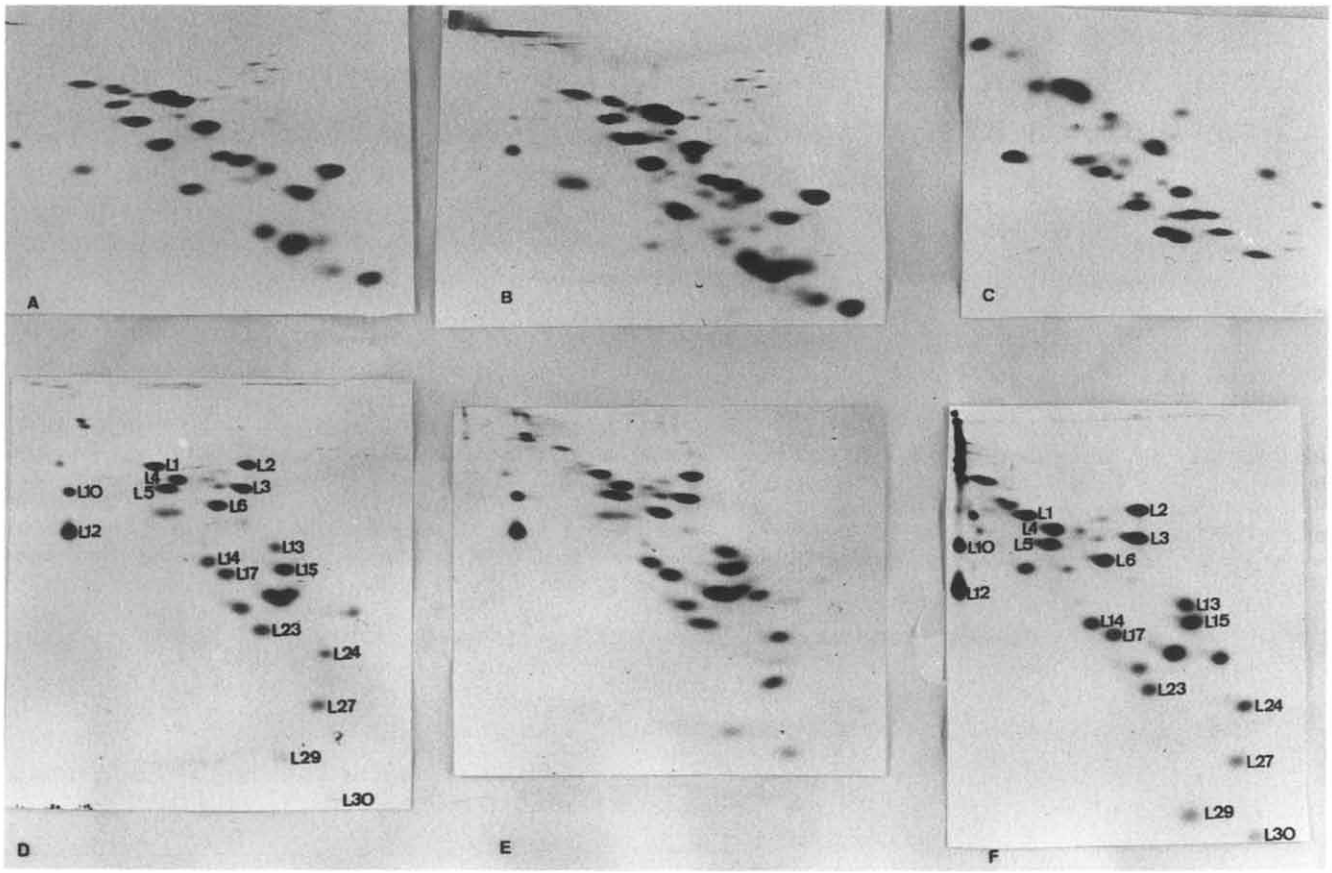

Fig. 1. 2D patterns for ribosomal proteins from *B. caldolyticus* and *B. stearothermophilus*. Gels are shown for the 30 S and 50 S proteins, respectively, for *B. caldolyticus* (A) and (D), comigration of *B. stearothermophilus/B. caldolyticus* (B) and (E), and for *B. stearothermophilus* (C) and (F).

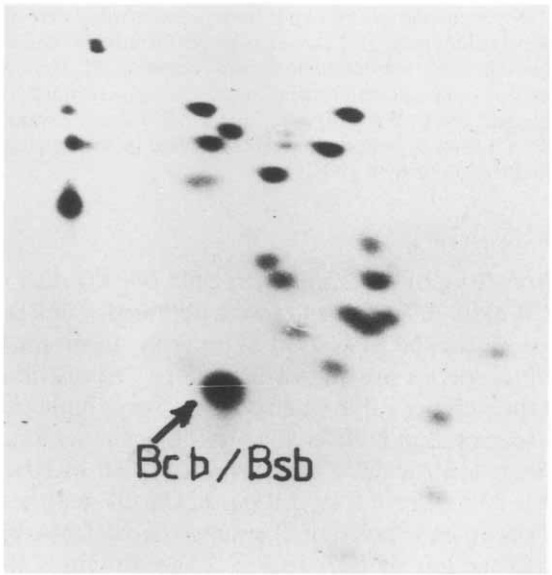

Fig. 2. 2D patterns of DNA bp II: co-migration of the *B. caldolyticus* (Bcb) and *B. stearothermophilus* (Bsb) proteins. 50 S ribosomal proteins from *B. caldolyticus* are added as a reference background.

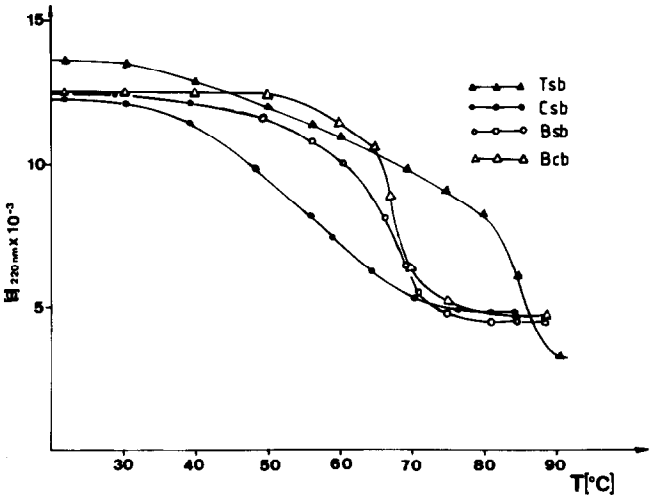

Fig. 3. Thermal denaturation of the DNA bp II of *B. caldolyticus* (Bcb), *B. stearothermophilus* (Bsb), *C. pasteurineum* (Csb), and *Th. thermophilus* (Tsb) as followed by the circular dichroism at 220 nm.

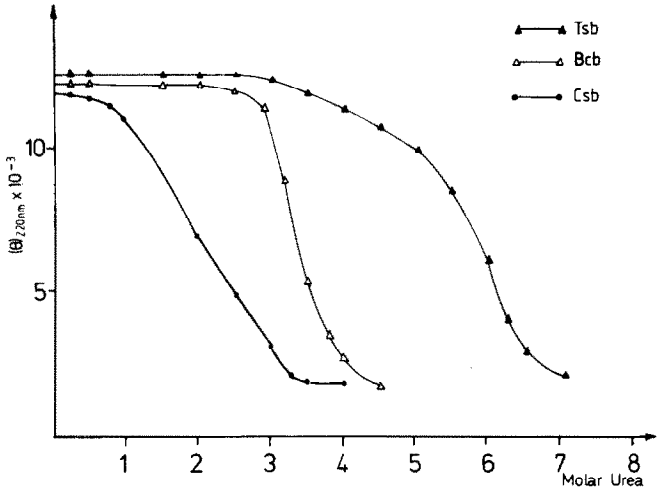

Fig. 4. Urea denaturation of DNA bp II of *Th. thermophilus* (Tsb), *B. caldolyticus* (Bcb), and *C. pasteurineum* (Csb) as followed by the circular dichroism at 220 nm.

from two *Bacilli* occupy identical positions on the 2D. The patterns of tryptic peptides on the fingerprints from the two DNA bp II are very similar and the same sequence as for *B. stearothermophilus* peptides was found (data not shown). The thermal (Fig. 3) and urea (Fig. 4) denaturation of DNA bp II from the two *Bacilli* yielded melting curves which were closely similar. The melting profile of the homologous proteins from *C. pasteurineum* (Csb) and *Th. thermophilus* (Tsb) are shown as representatives for mesophilic and extreme thermophilic prokaryotes. In filter binding assays the protein retained approximately 20% of the labelled DNA on the filter. This level of binding is also found for other members of the DNA bp II family [4].

The NMR spectra (data not shown) of native, urea denatured and oligodeoxynucleotide (dA_8/T_8) bound DNA bp II are identical to the spectra obtained using the *B. stearothermophilus* protein [4]. The binding of the Bcb protein to plasmid DNA RSF 1010 (Fig. 5) in-

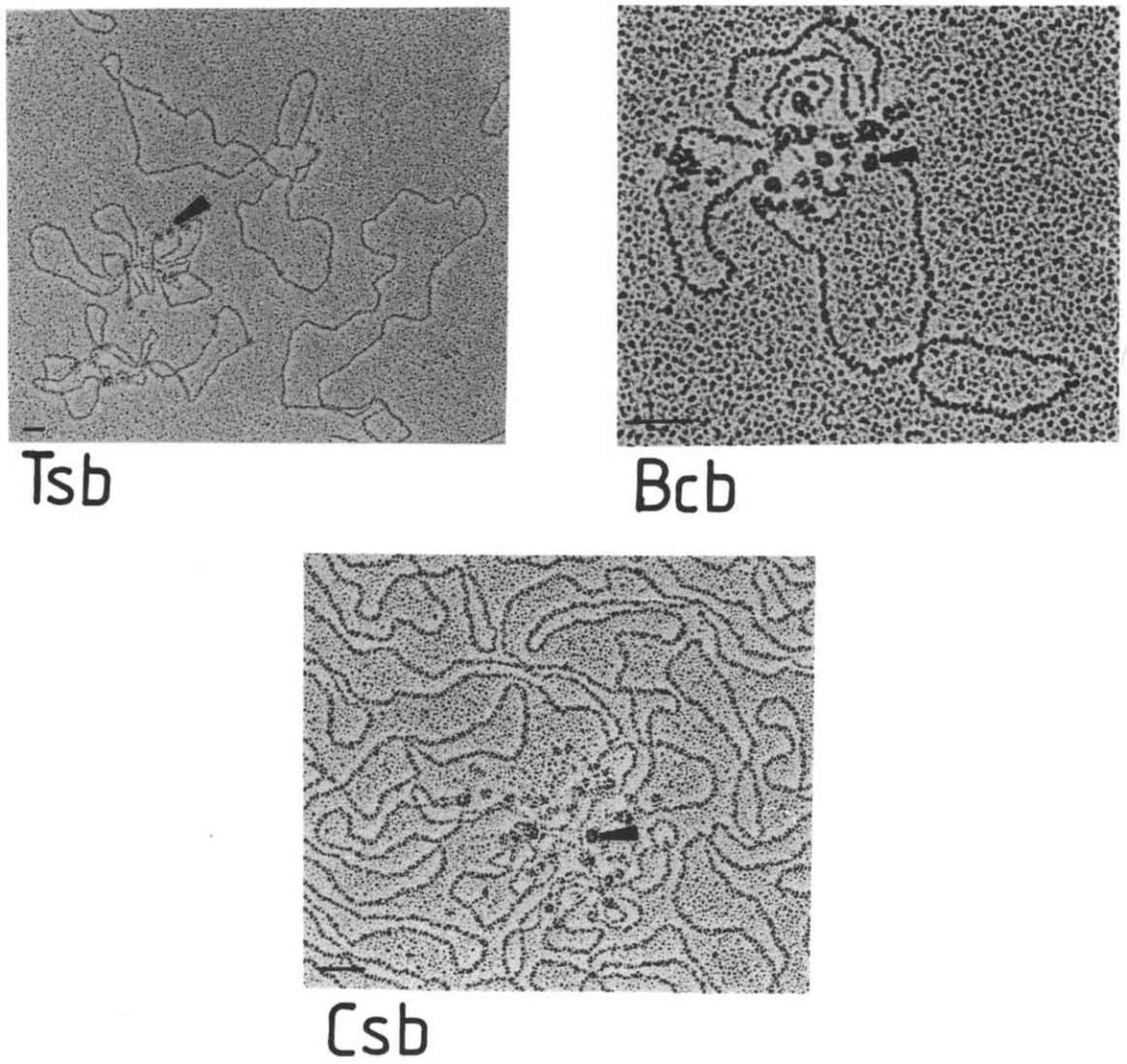

Fig. 5. Electron micrograph of the DNA bp II complexed with plasmid RSF 1010 DNA from *Th. thermophilus* (Tsb), *B. caldolyticus* (Bcb) and *C. pasteurineum* (Csb). Bar = 100 nm.

troduces the bead-like structures as shown for the homologous proteins from *Th. thermophilus* (Tsb) and *C. pasteurineum* (Csb). Crystals of DNA bp II from *B. caldolyticus* were obtained under the same conditions as for *B. stearothermophilus* [4] (data not shown). The crystals were too small for X-ray analysis and appeared identical to those obtained for the protein from *B. stearothermophilus*.

4. DISCUSSION

The use of thermophilic bacteria as a source of ribosomal and DNA bp II for crystallization has proven to be profitable [13]. This study of the proteins from *B. caldolyticus* was begun at a time when the analysis of the *B. stearothermophilus* proteins was in the early stages and the crystals of the proteins had, at that time, not been grown large enough or reproducibly enough for crystallographic analysis. We chose to investigate the *B. caldolyticus* proteins in the hope that their policy of trying other species, may well lead to improved crystals.

Two characteristic features which are easily accessible and are relevant to our current projects are the 2D pattern of ribosomal proteins and the properties of DNA bp II. The latter is an abundant and ubiquitous protein in prokaryotes, easily prepared in pure form [4]. The amino acid sequences of the DNA bp II from the two *Bacilli* appear to be identical, or at least extremely similar.

The DNA binding properties of the *B. caldolyticus* bp II in solution show that the protein induces the characteristic bead-like structures in DNA and binds non-specifically not only to DNA and to deoxynucleotides but also to rRNA and ribosomal subunits. This is different from the binding properties of the two species of DNA bp II from *E. coli* which preferentially bind to the native 30 S subunit [14].

In conclusion, *B. caldolyticus* strain EP 00275 grown at 65°C is close to identical to *B. stearothermophilus*, in the structure of its ribosomal and DNA bp II. Proteins from the strain of *B. caldolyticus* used here do not possess extra thermal stability over those from *B. stearothermophilus* or a significant variation in amino acid sequence. These results are in accord with a phenotypic and genotypic characterization of these *Bacilli* [15].

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