Biologia, Bratislava, **59**/3: 391—398, 2004

Cloning and biochemical characterization of DivIVA protein from *Bacillus stearothermophilus*

Ľudmila Pribišová, Katarína Muchová & Imrich Barák*

Institute of Molecular Biology, member of the Centre of Excellence for Molecular Medicine, Slovak Academy of Sciences, Dúbravská cesta 21, SK-84551 Bratislava 45, Slovakia; phone: ++ 421 2 5930 7418, fax: ++ 421 2 5930 7416, e-mail: imrich.barak@savba.sk

Pribišová, Ľ., Muchová, K. & Barák, I., Cloning and biochemical characterization of DivIVA protein from *Bacillus stearothermophilus*. Biologia, Bratislava, **59:** 391—398, 2004; ISSN 0006-3088. (Biologia). ISSN 1335-6399 (Biologia. Section Cellular and Molecular Biology).

DivIVA is a bifunctional protein involved in division site selection, by directing the activity of MinCD complex during vegetative growth of *Bacillus subtilis* cell. Its second functional role is in segregation of the chromosome during early stages of sporulation process. The *divIVA*-like genes are present in many bacteria, however, the role of most of these DivIVA homologues is not well documented. We have studied DivIVA homologue from *Bacillus stearothermophilus* ATCC 12980 strain. The identified *divIVA* gene was cloned and sequenced. DivIVA protein was expressed in *Escherichia coli*, purified and characterized. Computer analysis of the secondary structure of *B. stearothermophilus* DivIVA homologue indicated the α-helical coiled-coil structure. Gel filtration experiments showed that *B. stearothermophilus* DivIVA oligomerizes similarly as DivIVA from *B. subtilis*.

Key words: bacterial cell division, DivIVA, *Bacillus subtilis*.

Introduction

The first step in binary fission of *E. coli* as well as *B. subtilis* is the formation of the Z-ring. This ring is formed by polymerization of FtsZ protein. However, it has been shown that regulation of initiation of the synthesis of the Z-ring in *E. coli* is distinct from initiation of the synthesis in *B. subtilis*.

Precise localization of this ring in the middle of the cell during vegetative growth in *E. coli* is ensured by the MinCDE system, which consists of three proteins MinC, MinD and MinE. MinC inhibits polymerization of FtsZ (Hu et al., 1999) and is active only in complex with MinD. The Min proteins are organized into extended membrane-associated coiled structures, which are periodically assembled and disassembled during the pole-to-pole oscillation cycle (Shih et al., 2003). Concentration of MinCD is negatively affected by MinE, which is present in the middle of the cell in the highest concentration. The amount of MinCD sufficient to inhibit the FtsZ polymerization is present only at the cell poles. MinE thus acts as a topological marker for the Z-ring formation; the Z-ring is formed in the middle of the cell (Raskin & deBoer, 1999, Fu et al., 2001).

In *B. subtilis*, there is the MinCD/DivIVA system to ensure precise localization of the Z-ring during vegetative growth. MinC and MinD proteins are homologous to *E. coli* MinC and MinD. Their localization to the polar sites seems to be

* Corresponding author

mediated through DivIVA protein, which does not exhibit any sequence or structural similarity with MinE protein. It seems that DivIVA protein performs the same function as MinE but by a fully different mechanism (Cha & Stewart, 1997; Edwards & Errington, 1997).

DivIVA is targeted to the new division sites in late step in their assembly, and it remains at the new cell pole after division is completed (Marston & Errington, 1999). Similar pattern of localization of *B. subtilis* DivIVA was observed when *divIVA* was cloned into *E. coli* and also into yeast *Schizosacharomyces pombe* cells, in spite of a different mechanism of division (Edwards et al., 2000). Recently, it was shown that DivIVA accumulates at the cell poles of outgrowing spores and thus it seems that its localization is independent of all other division proteins (Hamoen & Errington, 2003).

DivIVA protein ensures that MinCD specifically inhibits division at the cell poles and allows division at the mid-cell during vegetative growth (Cha & Stewart, 1997; Edwards & Errington, 1997). During sporulation, when the localization of the division septum must be switched from the mid-cell to polar site, the MinCD/DivIVA system must somehow allow the formation of a polar Z-ring. Although the mechanism of DivIVA action during asymmetric septum formation is not known, Thomaides et al. (2001) showed that DivIVA interacts with the chromosome segregation machinery and helps to localize the *oriC* region of the chromosome near the cell pole during sporulation. This interaction is probably interposed by Soj-Spo0J-RacA system. Spo0J is a protein associated with the chromosome, it specifically binds around the *oriC* region. Soj protein facilitates movement of the Spo0J/*oriC* complex towards the cell pole. RacA also binds to the chromosome around the *oriC* and attaches this region to the pole by possible interaction with DivIVA (Wu & Errington, 2003). Switch of the division septum from the mid-cell to polar site is probably performed by dual function of DivIVA protein. It is responsible for sequestering MinCD at the poles during vegetative growth and RacA during sporulation (Ben-Yehuda et al., 2003).

Role of DivIVA in cell division, nucleoid segregation and growth inhibition was observed in Gram-positive bacterium *Streptococcus pneumoniae* (Fadda et al., 2003). DivIVA homologue in *Streptomyces coelicolor* has a function related to asymmetrical growth and cell shape determination (Flardh, 2003).

B. subtilis DivIVA protein displays a weak sequence similarity to eukaryotic tropomyosin (Edwards et al., 2000) forming dimers in solution. The predicted structure of *B. subtilis* DivIVA protein suggested an α-helical coiled-coil structure that is characterized by repeating heptad motif of amino acids being predominantly hydrophobic at the first and the fourth positions in each heptad and hydrophilic at the further positions (Lupas et al., 1991; Edwards et al., 2000). These repeating motives are probably used for oligomerization as was shown for tropomyosin (Lupas, 1996). Oligomerization of DivIVA in *B. subtilis* cell lysates was confirmed by Western blot analysis of non-denaturating polyacrylamide gels (Muchová et al., 2002a). Similar results were obtained by electron microscopy of *B. subtilis* DivIVA. It has been shown that protein can form oligomers consisting of up to 6–8 monomers (Stahlberg et al., 2004).

We have previously performed crystallization trials with *B. subtilis* DivIVA protein (Muchová et al., 2002b). However, the obtained crystals either did not diffract or diffracted weakly. Because of the experience that the thermophilic proteins are more suitable for crystallization trials than mesophilic proteins, we decided to use DivIVA homologue from *B. stearothermophilus* ATCC 12980 in order to increase the chance for successful crystallization. We have obtained *B. subtilis* DivIVA homologue from *B. stearothermophilus* ATCC 12980. Using alignment of *B. subtilis divIVA* and sequenced part of *B. stearothermophilus* DSM 13240 chromosome retrieved from the website http://www.genome.ou.edu/bstearo.html, we identified *divIVA* homologue in this thermophilic microorganism. Putative *divIVA* gene was cloned and DNA sequenced. Interestingly, DNA sequencing of this gene revealed high degree of differences between both *B. stearothermophilus* strains. The computer analysis of deduced amino acid sequence suggested coiled-coil structure. This DivIVA homologue was overproduced in *E. coli* and purified. Western blot analysis of non-denaturating polyacrylamide gel of *B. stearothermophilus* DivIVA protein and gel filtration experiment showed that it oligomerizes in a similar way as *B. subtilis* DivIVA (Muchová et al., 2002a).

Material and methods

Bacterial strains and culture media

All bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strains were routinely grown in Luria-Bertani (LB) broth (Ausubel et. al., 1987). *E. coli* competent cells were grown in SOB medium

Table 1. Bacterial strains and plasmids used in this study.

Strain and plasmid	Genotype or relevant characteristics	Source or reference
Strains		
E. coli MM294	*endA1 hsdR17 supE44 thi/1 recA*$^+$	BACKMAN et al. (1976)
E. coli BL21(DE3)	*F-ompT hsdS*$_B$($r_B^- m_B^-$)gal dcm	Novagen
B. stearothermophilus ATCC 12980		NAZINA et al. (2001)
IB 880	pETIVA1 in *E. coli* MM 294	This work
IB 881	pETIVA2 in *E. coli* MM 294	This work
IB 882	pETIVA1 in *E. coli* BL 21 (DE3)	This work
Plasmids		
pET15b(+)	Amp^R, *lacI*, T7 promoter	Novagen
pET26b(+)	Kan^R, *lacI*, T7 promoter	Novagen
pETIVA1	Amp^R, *lacI*, T7 promoter, *divIVA*	This work
pETIVA2	Kan^R, *lacI*, T7 promoter, *divIVA*	This work

(AUSUBEL et. al., 1987). Selection of *E. coli* cells transformed with pETIVA1 and pETIVA2 was achieved by cultivation in the presence of ampicillin (100 μg/mL) or kanamycin (30 μg/mL) in agar or liquid medium, respectively.

Cloning of divIVA into pET vectors

B. stearothermophilus (ATCC 12980) chromosomal DNA was isolated according to the method by Cutting & Vander-Horn (1990) and used as a template in PCR amplification. *B. stearothermophilus* divIVA was amplified by PCR using:

upstream primer: 5'-GAGGTGGCCCATATGCCGTT GACGCCATTGGAT-3'

downstream primer: 5'-GGCGGCGGATCCGGCGAT TTTTGCCAAT-3'

containing the underlined *Nde*I (upstream primer) and *Bam*HI (downstream primer) restriction sites. Reaction mixture (50 μL) contained 100 pmol upstream and downstream primers, 1 μg template chromosomal DNA, 10 nmol dNTP and 0.5 μL DNA Taq polymerase (0.5 U) (Finenzyme). PCR was carried out as follows: 94 °C for 1min, 39 °C for 1 min and 72 °C for 2 min in 30 cycles using an automatic thermal cycler (Gene Amp PCR System 2400, Perkin-Elmer, Foster City, CA). PCR reaction product was analyzed on a 0.8% agarose gel. PCR fragment was isolated from gel using Promega Wizard^{TM} PCR Preps DNA purification system. To prepare pETIVA1 and pETIVA2, the isolated PCR fragment coding for the *B. stearothermophilus divIVA* was digested with *Nde*I and *Bam*HI and ligated into *Nde*I/*Bam*HI cut pET15b(+) and pET26b(+) plasmids downstream of the IPTG inducible T7 promoter. Ligation mixture was used for transformation of *E. coli* MM294. Transformation of *E. coli* strain was performed by standard technique (AUSUBEL et al., 1987). Recombinant plasmids were verified by digestion with series of restriction endonucleases and subsequently DNA sequenced.

Overproduction and purification of B. stearothermophilus DivIVA

E. coli strain BL21 (DE3) was used for production of recombinant *B. stearothermophilus* DivIVA protein. This strain was transformed with recombinant plasmid pETIVA1 to generate strain IB 882. Cell culture was grown in LB medium supplemented with ampicillin (100 μL/mL) at 37 °C. When cell culture reached OD_{600} of 0.6, expression of His_6Tag-DivIVA was induced by the addition of IPTG to a final concentration of 1mM. After 5 hours of further growth at 37 °C, cells were harvested by centrifugation and pellet was frozen at −70 °C until use. Expression of DivIVA was confirmed by 12.5% SDS-PAGE analysis. Cells were resuspended on ice in binding buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM AEBSF), lyzed by sonication and centrifuged at 30 000 rpm for 30 min. To purify His_6Tag-DivIVA, metal chelate affinity chromatography was used. The soluble fraction was applied onto a 2 mL Ni^{2+}-NTA column (Amersham Pharmacia) previously preequilibrated in binding buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl). His_6Tag-DivIVA was eluted with step gradient of a 0.1 M, 0.2 M, 0.3 M and 1 M imidazol, which bound to the Ni-NTA and displaced the tagged protein. Fraction of 0.2 M imidazol containing His_6Tag-DivIVA was applied onto 1 mL Sephadex G-25 column preequilibrated in buffer B (10 mM Tris-HCl pH 8.0, 100 mM NaCl) and His_6Tag-DivIVA protein was eluted with buffer B. The His_6Tag-DivIVA was loaded onto Superose 12 gel filtration column and eluted with buffer A (50 mM Tris-Cl pH 8.0, 100 mM NACl, 1 mM EDTA).

DNA sequencing

The *divIVA* gene in pETIVA1 and pETIVA2 were sequenced by dideoxynucleotide chain termination procedure (SANGER et al., 1977) using T7 sequencing primers flanking *divIVA*. Nucleotide sequences were further analyzed using Vector NTI suite 6.

SDS-PAGE and Western blot analysis

SDS-PAGE electrophoresis was performed according LAEMLI et al. (1970). Western blot analysis of the re-

combinant protein was performed using the semi-dry method (Hirano & Watanabe, 1990). Proteins after electrophoresis through a blue native 5–18% linear gradient polyacrylamide gel (Schagger et al., 1994) were blotted onto a nitrocellulose membrane. The *B. stearothermophilus* DivIVA was detected by incubation with the primary mouse polyclonal serum against *B. subtilis* DivIVA diluted 1:1000 followed by alkaline phosphatase-conjugated anti-mouse immunoglobulin G diluted 1:1000.

Computational analysis

Deduced amino acid sequence of *B. stearothermophilus* DivIVA was analyzed using services on ExPASy molecular biology server (Gasteiger et al., 2003; http://ca.expasy.org). Secondary structure prediction of the protein was performed with nnPREDICT (Kneller et al., 1990; http://www.cmpharm.ucsf.edu/~nomi/nnpredict.html) and PSA (Stultz et al., 1993; http://bmerc-www.bu.edu/psa/) programs. Putative coiled-coil structure was analyzed by COILS (Lupas et al., 1991; http://www.ch.embnet.org/software/COILS_form.html) and SMART (Letunic et al., 2002; http://smart.embl-heidelberg.de) programs. For determination of sequence similarity of predicted peptide sequence BLASTp (Altschul et al., 1990; http://www.ncbi.nlm.nih.gov/BLAST/) and MAXHOM (Sander & Schneider, 1991; http://cubic.bioc.columbia.edu/) programs were used.

Nucleotide sequence accession number

The DNA sequence data reported here have been submitted to the GenBank (Benson et al., 2004) and assigned accession number AY533033.

Results and discussion

Cloning of the B. stearothermophilus divIVA gene
PCR primers were designated according to the *divIVA* sequence of *B. stearothermophilus* DSM 13240 (http://www.genome.ou.edu/bstearo.html) from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) (Fig. 1). The *divIVA* gene from *B. stearothermophilus* ATCC 12980 was amplified by PCR. Obtained single fragment of expected 580 bp size was used for preparation of expression vectors pETIVA1 and pETIVA2. Amplified *divIVA* gene was cloned into pET15b(+) and pET26b(+) expression vectors using *Nde*I and *Bam*HI restriction sites. Two recombinant clones, which were obtained after transformation of *E. coli* MM294 with ligation mixtures, were chosen to be DNA sequenced.

Analysis of the B. stearothermophilus divIVA gene
The two fragments, 627 bp of pETIVA1 and 632 bp of pETIVA2, were DNA sequenced. pETIVA1 plasmid contained *B. stearothermophilus divIVA* gene encoding 190 amino acid residues of His_6-tagged DivIVA. This DivIVA protein was used in gel filtration experiment and Western blot analysis. The *divIVA* gene in pETIVA2 plasmid encoded 170 amino acid residues of DivIVA. The DNA sequences of open reading frames of *divIVA* from pETIVA1 and pETIVA2 were compared. Their comparison confirmed 100% identity of these sequences. Thus, we propose that the clones, which we obtained after transformation of *E. coli* strain MM294, had no mutations in the *divIVA* gene. In contrast, when the similar experiments were carried out with *divIVA* gene from *B. subtilis*, the authors obtained mutations and deletions in the *divIVA* gene in each clone (Muchová et al., 2002a). It is very likely that DivIVA from *B. subtilis* can interact with the *E. coli* division apparatus in a way that inhibits growth because *B. subtilis* DivIVA protein is targeted to the division sites in *E. coli* (Edwards et al., 2000). It is also probable that point mutations and deletions in *divIVA* gene are selected allowing the *E. coli* host strain to escape the negative effect of *B. subtilis* DivIVA on cell growth. However, this effect was not observed for *B. stearothermophilus* DivIVA. When we compared DivIVA from *B. stearothermophilus* with DivIVA from *B. subtilis*, alignment revealed relatively low degree of homology: 66.8% identity in nucleotide sequences (Fig. 1) and 66.5% identity in amino acid sequences (Fig. 2). We suggest that *B. stearothermophilus* DivIVA interacts with *E. coli* division apparatus differently than *B. subtilis* DivIVA does.

Interestingly, DNA sequence identity between the *divIVA* from *B. stearothermophilus* ATCC 12980 and from *B. stearothermophilus* DSM 13240 is only 86.4% (Fig. 1). The proteins predicted from the open reading frames share, however, 94.7% identity (Fig. 2).

Expression and purification of recombinant DivIVA
To overexpress His_6 Tag-DivIVA, *E. coli* BL21(DE3) strain transformed with pETIVA1 plasmid was used. The highest expression of DivIVA after induction with IPTG was obtained after five hours. Analysis of expression of DivIVA in *E. coli* cells showed that the protein was present both in the soluble fraction and cell pellet (Fig. 3A). The amount of protein in soluble fraction of cell lysate

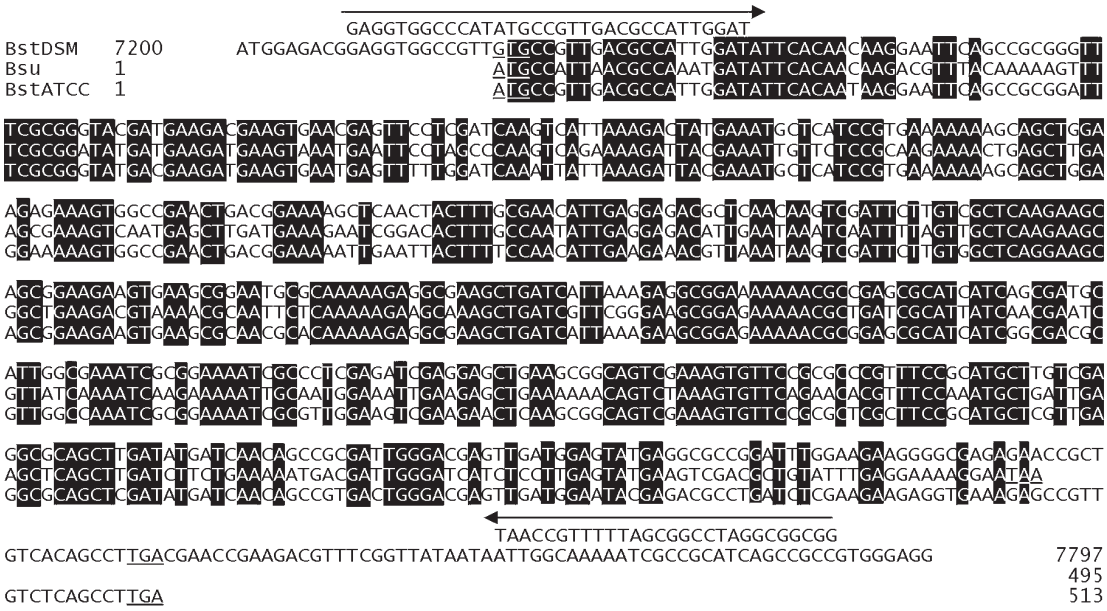

Fig. 1. Comparison of DNA of a segment of contig 619 from sequenced *B. stearothermophilus* DSM 13240 (BstDSM), *divIVA* gene from *B. subtilis* KSS1168 (Bsu) and *divIVA* gene from *B. stearothermophilus* ATCC 12980 (BstATTC). Sequence identity between the *divIVA* from *B. stearothermophilus* ATCC 12980 and those from *B. stearothermophilus* DSM 13240 and *B. subtilis* is 86.4% and 66.8%, respectively. The start and stop codons are underlined. The oligonucleotide primers used for PCR are marked.

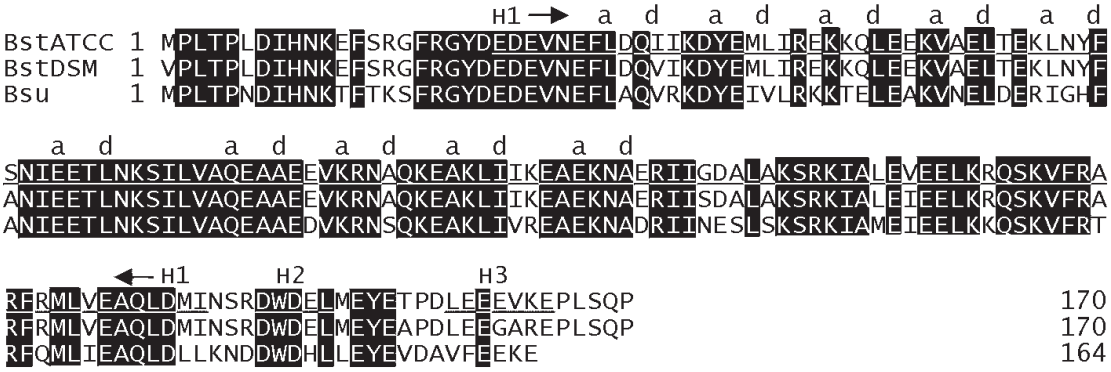

Fig. 2. Comparison of amino acid sequences of DivIVA: *B. stearothermohilus* DSM 13240 (BstDSM), *B. stearothermophilus* ATCC 12980 (BstATCC) and *B. subtilis* (Bsu). H1, H2 and H3 denote three predicted α-helices. Letters *a* and *d* denote predominantly hydrophobic amino acids at first and fourth position in predicted heptad repeats. Sequence identity between the DivIVA from *B. stearothermophilus* ATCC 12980 and those from *B. stearothermophilus* DSM 13240 and *B. subtilis* is 94.7% and 66.5%, respectively.

was sufficient, therefore the recombinant protein was purified from soluble fraction of cell lysate. Most efficient elution of the His-tagged protein from the Ni-column was achieved by 0.2 M and 0.3 M imidazole (Fig. 3B). Sephadex G-25 column was used for buffer exchange. The protein yield from the soluble fraction of the cell lysate was about 3 mg purified protein per 50 mL of cell culture.

Oligomerization of B. stearothermophilus DivIVA

The purified His_6Tag-DivIVA protein (molecular weight of monomer is 22.04 kDa) was loaded

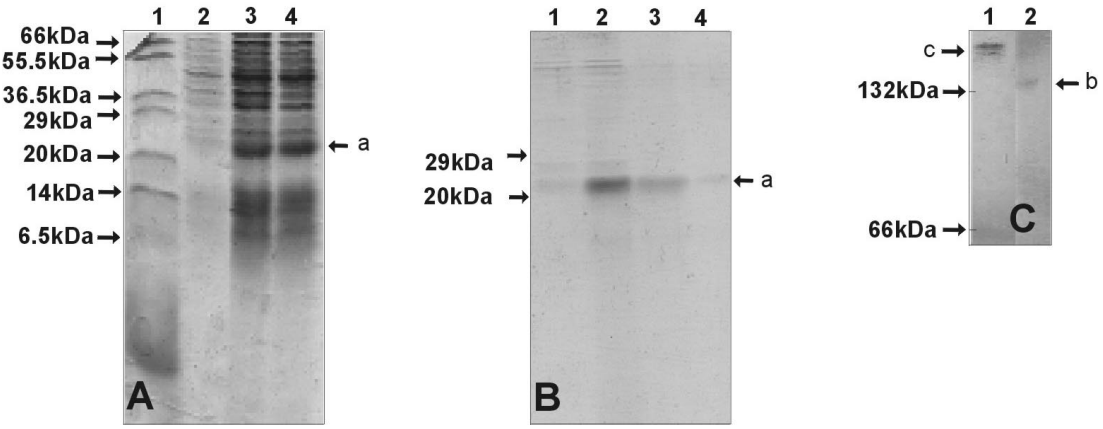

Fig. 3. Expression, purification and oligomer formation analysis of DivIVA protein. a – His_6Tag-DivIVA *from B. stearothermophilus*, b – *B. stearothermophilus* His_6Tag-DivIVA oligomer, c – *B. subtilis* DivIVA oligomer. (**A**) 15% SDS-PAGE analysis of expression of DivIVA. Lane 1, molecular mass marker; lane 2, total cell protein from uninduced *E. coli* BL21 (DE3) with pETIVA1; lane 3, soluble fraction of cell lysate from induced *E. coli* BL21 (DE3) harboring pETIVA1; lane 4, cell pellet from induced *E. coli* BL21 (DE3) harboring pETIVA1. (**B**) 12.5% SDS-PAGE analysis of elution the His_6-tagged DivIVA from Ni-NTA-column. Lanes 1-4, fractions eluted by 0.1 M, 0.2 M, 0.3 M, 1.0 M imidazol, respectively. (**C**) Western blot analysis of blue native gel of *B. stearothermophilus* DivIVA protein. Lane 1, *B. subtilis* DivIVA; lane 2, *B. stearothermophilus* ATCC 12980 DivIVA.

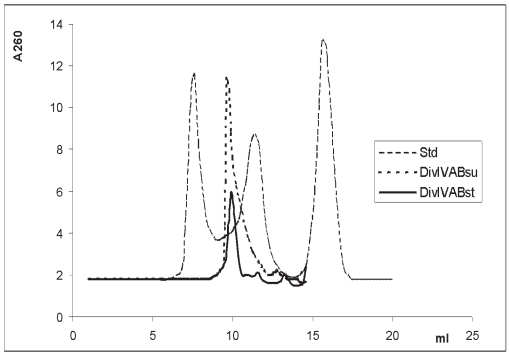

Fig. 4. Superose 12 gel filtration of *B. subtilis* DivIVA and His_6-tagged *B. stearothermophilus* DivIVA. 0.5 mL of *B. subtilis* DivIVA after FastQ and MonoQ ion exchange columns was loaded. 0.5 mL of *B. stearothermophilus* DivIVA after Ni-NTA-column and Sephadex G-25 column was loaded. Chromatogram of molecular weight standards: Blue dextran (2,000 kDa), aldolase (158 kDa) and cytochrome *c* (12.5 kDa).

onto Superose 12 gel filtration column. The retention volume of His_6Tag-DivIVA (*B. stearothermophilus* DivIVA) protein was between the molecular weight standards of the 158 kDa and 2,000 kDa (Fig. 4). The same result was obtained for *B. subtilis* DivIVA (molecular weight of monomer is 19.5 kDa) and *B. subtilis* His_6Tag-DivIVA (molecular weight of monomer is 21.5 kDa). Based on this result and assuming that *B. stearothermophilus* DivIVA forms a similarly shaped oligomers, we can suggest that thermophilic DivIVA forms oligomers with a similar molecular weight as *B. subtilis* DivIVA oligomers. Oligomerization of *B. subtilis* DivIVA was confirmed by analytical ultracentrifugation (Muchová et al., 2002a). These observations showed existence of various oligomeric species with the molecular mass averages extended from values associated with a dimer to that associated with at least a decamer. Recently, the ability of *B. subtilis* DivIVA to form "doggy bone" shaped particles was observed. They were interpreted as DivIVA 6-8 mers (Stahlberg et al., 2004). Thus, we would like to propose that *B. stearothermophilus* DivIVA forms 6-8 mers similarly as *B. subtilis* DivIVA.

Oligomerization of *B. stearothermophilus* DivIVA was also observed by Western blot analysis of non-denaturating polyacrylamide gel (Fig. 3C). The positions of signals for *B. stearothermophilus* and *B. subtilis* DivIVA oligomers were slightly different. However, it does not mean that they differ in quaternary structure. This observation can be caused by different interaction of Coomassie Briliant Blue (CBB) with both proteins. CBB interacts with all positively charged amino acids on the surface of a protein. The total charge of the protein is then characterized by its initial negative charge and by charge obtained from CBB.

Electrophoretic mobility of protein in blue native gel is dependent on the shape of molecule and on the charge of the protein complex. His_6-tagged *B. stearothermophilus* DivIVA has more positively charged amino acid residues than its *B. subtilis* counterpart and thus it has higher mobility in blue native gel.

Computational analysis of DivIVA sequence
Based on the results of nnPREDICT and PSA programs, secondary structure elements of *B. stearothermophilus* DivIVA were predicted. It is expected that the protein contains three α-helixes Glu22-Ile143, Arg147-Leu151 and Leu159-Glu165, marked as H1, H2 and H3 (Fig. 2). Analyses by the COILS program and the web-service SMART confirmed heptad repeat in the DivIVA sequence (Fig. 2) and predicted the start and the end of coiled-coil region at Phe28 and Ile103, respectively. These analyses show that the coiled-coil region is localized inside of H1. The similar localization of coiled-coil region with start at the N-terminus of protein sequence was determined in the *B. subtilis* DivIVA sequence (EDWARDS et al., 2000). Breaking of the α-helical structure by Leu120Pro (DivIVA2) mutation in the coiled-coil region leads to the change of ability to oligomerize, while Ala78Thr (DivIVA1) mutation had no effect on the DivIVA oligomerization (MUCHOVÁ et al., 2002a), although the latter mutation disrupts functional abilities of DivIVA.

Acknowledgements

The authors would like to thank Dr. Dušan PEREČKO for helpful discussion, Emília CHOVANCOVÁ and Helena KOLESÁROVÁ for technical assistance. This work was supported by the grant No. 2/1004/21 from the Slovak Academy of Sciences and The Wellcome Trust Grant 066732/Z/01/Z.

References

ALTSCHUL, S. F., GISH, W., MILLER, W., MYERS, E. W. & LIPMAN, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

AUSUBEL, F. M., BRENT, R., KINGSTON, R. E., MOORE, D. O., SEIDMANN, J. S., SMITH, J. & STRUHL, K. 1987. Current Protocols in Molecular Bioloy. John Wiley & Sons, Ltd., New York.

BACKMAN, K., PTASHNE, M. & GILBERT, A. W. 1976. Construction of plasmids carrying the *cl* gene of bacteriophage lambda. Proc. Natl. Acad. Sci. USA **73:** 4174–4178.

BENSON, D. A., KARSCH-MIZRACHI, I., LIPMAN, D. J., OSTELL, J. & WHEELER, D. L. 2004. GenBank: update. Nucleic Acids Res. 2004 **32:** D23–D26.

BEN-YEHUDA, S., RUDNER, D. Z. & LOSICK, R. 2003. RacA, a bacterial protein that anchors chromosomes to the cell poles. Science **299:** 532–536.

CHA, J., & STEWART, G. C. 1997. The *divIVA* minicell locus of *Bacillus subtilis*. J. Bacteriol. **179:** 1671–1683.

CUTTING, S. M. & VANDER-HORN, P. B. 1990. Genetic analysis, pp. 27–74. In: Harwood, C. R. & Cutting, S. M. (eds) Molecular Biological Methods for *Bacillus*. John Wiley & Sons, Ltd., Chichester, England.

EDWARDS, D. H. & ERRINGTON, J. 1997. The *Bacillus subtilis* DivIVA protein targets to the division septum and controls the site specificity of cell division. Mol. Microbiol. **24:** 905–915.

EDWARDS, D. H., THOMAIDES, H. B. & ERRINGTON, J. 2000. Promiscuous targeting of *Bacillus subtilis* cell division protein DivIVA to division sites in *Escherichia coli* and fission yeast. EMBO J. **19:** 2719–2727.

FADDA, A., PISCHEDDA, C., CALDARA, F., WHALEN, M. B., ANDERLUZZI, D., DOMENICI, E. & MASSIDDA, O. 2003. Characterization of *divIVA* and other genes located in the chromosomal region downstream of the *dcw* cluster in *Streptococcus pneumoniae*. J. Bacteriol. **20:** 6209–6214.

FLARDH, K. 2003. Essential role of DivIVA in polar growth and morphogenesis in *Streptomyces coelicolor* A3(2) Mol. Microbiol. **49:** 1523–1536.

FU, X., SMIS, Y. L., ZHANG, Y. & ROTHFIELD, L. I. 2001. The MinE required for proper placement of the division site is a mobile structure that changes its cellular location during the *Escherichia coli*. Proc. Natl. Acad. Sci. USA **98:** 980–985.

GASTEIGER, E., GATTIKER, A., HOOGLAND, C., IVANYI, I., APPEL, R. D. & BAIROCH, A. 2003. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res. **31:** 3784–3788.

HAMOEN, L. W. & ERRINGTON, J. 2003. Polar targeting of DivIVA in *Bacillus subtilis* is not directly dependent on FtsZ or PBP 2B. J. Bacteriol. **185:** 693–697.

HIRANO, H. & WATANABE, T. 1990. Microsequencing of proteins electrotransferred on to immobilizing matrices from polyacrylamide gel electrophoresis: Application to an insoluble protein. Electrophoresis **11:** 573–580.

HU, Z. MUKHERJEE, A., PICHOFF, S. & LUTKENHAUS, J. 1999. The MinC component of the division site selection system in *Escherichia coli* interacts with FtsZ to prevent polymerization. Proc. Natl. Acad. Sci. USA **96:** 14819–14824.

KNELLER, D. G., COHEN, F. E. & LANGRIDGE, R. 1990. Improvements in protein secondary structure prediction by an enhanced neural network. J. Mol. Biol. **214:** 171–182.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the bacteriophage T4. Nature **227:** 680–685.

Letunic, I., Goodstadt, L., Dickens, N. J., Doerks, T., Schultz, J., Mott, R., Ciccarelli, F., Copley, R. R., Ponting, C. P. & Bork, P. 2002. Recent improvements to the SMART domain-based sequence annotation resource. Nucleic Acids Res. **30:** 242–244.

Lupas, A. 1996. Prediction and analysis of coiled-coil structures. Methods Enzymol. **266:** 513–525.

Lupas, A., Van Dyke, M. & Stock, J. 1991. Predicting coiled coils from protein sequences. Science **252:** 1162–1164.

Marston, A. L. & Errington, J. 1999. Selection of the midcell division site in *Bacillus subtilis* through MinD-dependent polar localization and activation of MinC. Mol. Microbiol. **33:** 84–96.

Muchová, K., Kutejová, E., Scott, D. J., Brannigan, J. A., Lewis, R. J., Wilkinson, A. J. & Barák, I. 2002a. Oligomerization of the *Bacillus subtilis* division protein DivIVA. Microbiology **148:** 807–813.

Muchová, K., Kutejová, E., Pribišová, L., Wilkinson, A. J. & Barák, I. 2002b. *Bacillus subtilis* division protein DivIVA-screen for stable oligomer state conditions. Acta Cryst. **D58:** 1542–1543.

Nazina, T. N., Tourova, T. P., Poltaraus, A. B., Novikova, E. V., Grigoryan, A. A., Ivanova, A. E., Lysenko, A. M., Petrunyaka, V. V., Osipov, G. A., Belyaev, S. S. & Ivanov, M. V. 2001. Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus*, *G. thermocatenulatus*, *G. thermoleovorans*, *G. kaustophilus*, *G. thermoglucosidasius* and *G. thermodenitrificans*. Int. J. Syst. Evol. Microbiol. **51:** 433–446.

Raskin, D. M. & De Boer, P. A. 1999. Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **96:** 4971–4976.

Sander, C. & Schneider, R. 1991. Database of homology-derived structures and the structural meaning of sequence alignment. Proteins **9:** 56–68.

Sanger, F., Nicklen, S. & Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

Shih, Y., Le, T. & Rothfield, L. 2003. Division site selection in *Escherichia coli* involves dynamic redistribution of Min proteins within coiled structures that extend between two cell poles. Proc. Natl. Acad. Sci. USA **100:** 7865–7870.

Schagger, H., Cramer, W. A. & Von Jagow G. 1994. Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis an isolation of membrane protein complexes by two-dimensional native electrophoresis. Anal Biochem. **217:** 220–230.

Stahlberg, H., Kutejová, E., Muchová, K., Gregorini, E., Lustig, Q., Muller, S. A., Olivieri, V., Engel, A., Wilkinson, A. J. & Barák, I. 2004. Oligomeric structure of the *Bacillus subtilis* cell division protein DivIVA determined by transmission electron microscopy. Mol. Microbiol. **52:** 1281–1290.

Stultz, C. M., White, J. V. & Smith, T. F. 1993. Structural analysis based on state-space modeling. Protein Sci. **2:** 305–314.

Thomaides, H. B., Freeman, M., Karoui, M. & Errington, J. 2001. Division site selection protein DivIVA of the *Bacillus subtilis* has a second distinct function in chromosome segregation during sporulation. Gen. Dev. **15:** 1662–1673.

Wu, L. J. & Errington, J. 2003. RacA and the Soj-Spo0J system combine to effect polar chromosome segregation in sporulating *Bacillus subtilis*. Mol. Microbiol. **49:** 1463–1475.

Received January 23, 2004
Accepted March 4, 2004