MvaT proteins in *Pseudomonas* spp.: a novel class of H-NS-like proteins

The H-NS protein was isolated for the first time from *Escherichia coli* as a contaminant in RNA polymerase preparations (Jacquet *et al.*, 1971). A few years later, on the basis of nucleotide sequence homology, H-NS proteins were identified by different groups in other enteric or related bacteria such as *Proteus mirabilis*, *Serratia marcescens*, *Salmonella typhimurium* and *Erwinia chrysanthemi* (Ussery *et al.*, 1994). Moreover, by *in silico* analysis of the first completely sequenced bacterial genome, an additional H-NS-related protein was detected in *Haemophilus influenzae* (Fleischmann *et al.*, 1995). Finally, a paralogous protein named StpA was found in both *Escherichia coli* and *Salmonella typhimurium* (Dorman *et al.*, 1999). All these proteins display more than 70 %

amino acid sequence identity, indicating that they belong to the same protein family.

Initially, H-NS proteins were thought to be restricted to members of the *Enterobacteriaceae* and related bacteria (Atlung & Ingmer, 1997), until the discovery of the first H-NS-like protein outside this group. Indeed, by biochemical methods, the so-called BpH3 protein was isolated from *Bordetella pertussis*, a non-enteric Gram-negative bacterium belonging to the *β-Proteobacteria*. The protein sequence shows only 40 % identity with the *E. coli* H-NS protein but is able to reverse H-NS deficiency in an *E. coli* mutant strain (Goyard & Bertin, 1997). Consequently, these data raised new perspectives concerning the existence of such proteins in phylogenetically distant bacterial species. Taking advantage of the serine susceptibility on minimal medium of an *E. coli hns* mutant strain, we recently isolated several H-NS-related proteins from various bacteria, for example, *Vibrio cholerae*, the agent of cholera disease (Tendeng *et al.*, 2000), *Yersinia enterocolitica*, responsible for gastroenteritis in humans (Bertin *et al.*, 2001), *Photorhabdus luminescens*, an entomopathogenic bacterium (Tendeng, 2002), and *Psychrobacter* TAD1, a psychrophilic micro-organism isolated in Antarctica (Tendeng *et al.*, 2003).

Except for enterobacteria, where H-NS has been shown to control the expression of many genes, for example, in *E. coli* (Hommais *et al.*, 2001), the role in bacterial physiology of most H-NS-related proteins remains unknown. However, they constitute a family of proteins displaying an evolutionarily conserved structural and functional organization in two modules (Dorman *et al.*, 1999; Bertin *et al.*, 1999). The N-terminal oligomerization domain displays strong amino acid sequence divergence but is predicted to adopt an α-helix structure in all H-NS proteins (Bertin *et al.*, 1999, 2001), consistent with the recent three-dimensional structure of this domain in H-NS of *E. coli* (Renzoni *et al.*, 2001; Bloch *et al.*, 2003). Unlike the N-terminal part, the C-terminal DNA-binding domain displays a strong consensus motif in the proteins characterized so far, with six strictly conserved residues Y-x(6)-[GS]-[ED]-x(0,2)-T-W-[TS]-G-[QR]-G-[RK]-x-P-x(4,5)-A-x(3,4)-G (Bertin *et al.*, 2001). These observations suggest that all H-NS-related proteins have the capacity to bind DNA via their C-terminal part and the propensity to form oligomers by their N-terminal domain. Both properties may explain why the proteins examined so far can restore the wild-type phenotype in an *E. coli hns* mutant strain (Bertin *et al.*, 1999, 2001).

To date, no H-NS protein has been characterized in Gram-positive bacteria or in archaea. Surprisingly, within Gram-negative bacteria, no H-NS-related protein has been identified in *Pseudomonas* strains, which, along with the enteric bacteria, belong to the *γ-Proteobacteria*. *Pseudomonas* spp. are known for their extreme ecological and metabolic diversity. Indeed, such bacteria have been collected from environments as diverse as soil, water (marine or freshwater), plants and animals, but their taxonomical classification is still a matter of controversy (Yamamoto *et al.*, 2000). Currently, the genome sequencing of six of them by different international consortia is underway (http://igweb.integratedgenomics.com/GOLD). The scanning of these bacterial genome sequences available in databases has not yet revealed the existence of any H-NS protein in *Pseudomonas* spp. We describe here for the first time the isolation and characterization of an *hns*-related gene from a psychrotrophic *Pseudomonas*, strain Y1000. Remarkably, its gene product does not contain the conserved consensus motif, in particular the strictly conserved amino acid residues, usually associated with the C-terminal domain of H-NS-like proteins (Bertin *et al.*, 2001).

To identify a protein functionally related to H-NS of *E. coli*, we constructed from *Pseudomonas* strain Y1000, collected from Lake Baikal (Soutourina *et al.*, 2001), a DNA genomic library that was used to complement the serine susceptibility on minimal medium of an *E. coli* H-NS-deficient mutant. Two hundred clones were obtained after incubation at 25 °C for 48 h on selective medium plates. About 30 clones were analysed for restriction profiles, and plasmid pDIA583 carrying a DNA fragment of 2000 bp was purified. Nucleotide sequence analysis of the insert revealed the presence of a complete coding sequence (CDS) of 366 bp, in the opposite orientation with respect to the resident *lac* promoter. A putative ribosome-binding site was identified 5 bp upstream from the ATG start codon. Minus 35 and −10 hexamers showing 67 % similarity with the σ^{70} consensus of *E. coli* separated by an 18 bp spacer were identified 49 bp upstream from this translational initiation codon. A motif reminiscent to the Y box recognized by nucleic-acid-binding protein, i.e. CAAAT, was identified 16 bp upstream from the ATG start codon. Interestingly, such a motif has also been identified as a putative cold-box upstream of the coding sequence of H-NS in *E. coli* (La Teana *et al.*, 1991) as well as in *V. cholerae* (Tendeng *et al.*, 2000) and *Y. enterocolitica* (Bertin *et al.*, 2001). The CDS encodes a putative protein of 121 aa with a predicted molecular mass of 13·614 kDa and a pI of 10·62 (EMBL accession no. AJ567347). Surprisingly, this putative protein showed no significant homology with any known H-NS-related protein (e.g. 18 % identity with the *E. coli* H-NS protein), even in the C-terminal domain that did not display the H-NS consensus sequence. Nevertheless, like the other members of the H-NS family, the N-terminal domain of the *Pseudomonas* strain Y1000 protein is predicted to be mainly α-helical and to adopt a coiled-coil conformation, while the C-terminal domain is a mixed α/β structure (data not shown). Moreover, using FROST, a fold-recognition method (http://www-mig.jouy.inra.fr), this domain was predicted to share the same three-dimensional structure as the *E. coli* H-NS C-domain resolved previously by NMR (Shindo *et al.*, 1995). Finally, the expression of the strain Y1000 protein structural gene in an *E. coli* mutant fully reversed additional *hns*-related phenotypes, for example, the loss of motility on semi-solid medium and the ability to use β-glucoside as a sole carbon source, as observed previously with other H-NS-like proteins (Goyard & Bertin, 1997; Bertin *et al.*, 1999, 2001; Tendeng *et al.*, 2000). This strongly suggests that the protein we characterized belongs to the H-NS family and shares the same structural and functional organization as members of this family despite the unusually low amino acid conservation.

The comparison of the *Pseudomonas* strain

Y1000 H-NS-like protein amino acid sequence with all sequences present in databases revealed approximately 50 % identity with MvaT, a transcriptional activator that participates in the control of mevalonate metabolism in *Pseudomonas mevalonii* (Rosenthal *et al.*, 1998). By scanning complete and unfinished bacterial genome sequences, we identified at least one homologous protein in each *Pseudomonas* species. Indeed, two putative proteins are present in *Pseudomonas aeruginosa*, three in *Pseudomonas fluorescens*, four in *Pseudomonas syringae* and five in *Pseudomonas putida*. In addition, an orthologous protein was identified in *Azotobacter vinelandii*, a bacterium belonging to the family *Pseudomonadaceae* (Fig. 1). All these proteins display at least 47 % amino acid identity. Except for the MvaT proteins of *P. mevalonii* and *P. aeruginosa*, which possess a regulatory role in mevalonate metabolism and in virulence gene expression, respectively (Rosenthal *et al.*, 1998; Diggle *et al.*, 2002), the function of the *Pseudomonas* H-NS-related proteins remains unknown and some have been annotated as hypothetical proteins. Overexpression of the *hns*-like gene of *Pseudomonas* strain Y1000 in the same strain from the low-copy-number vector pDIA584, i.e. a pBBR1MCS derivative (Kovach *et al.*, 1994) carrying this gene, resulted in a significant reduction in motility on semi-solid medium (12 mm of swarming ring at 25 °C as compared to 20 mm for the control with pBBR1MCS alone). This suggests that, like H-NS in *E. coli* (Bertin *et al.*, 1999; Soutourina *et al.*, 1999, 2001), the protein described here may play a role in the control of bacterial motility, at least in *Pseudomonas* strain Y1000. This is in accordance with the recently reported altered swarming ability of an *mvaT* mutant strain of *P. aeruginosa* (Diggle *et al.*, 2002).

Taken together, these observations suggest that, despite a low amino acid conservation, proteins structurally and functionally related to H-NS in *E. coli* are even more widespread than thought previously. The comparison of these proteins in terms of structure and function may help in the future to understand the properties of H-NS itself, in particular with respect to protein–protein interactions and DNA-binding specificity. More generally,

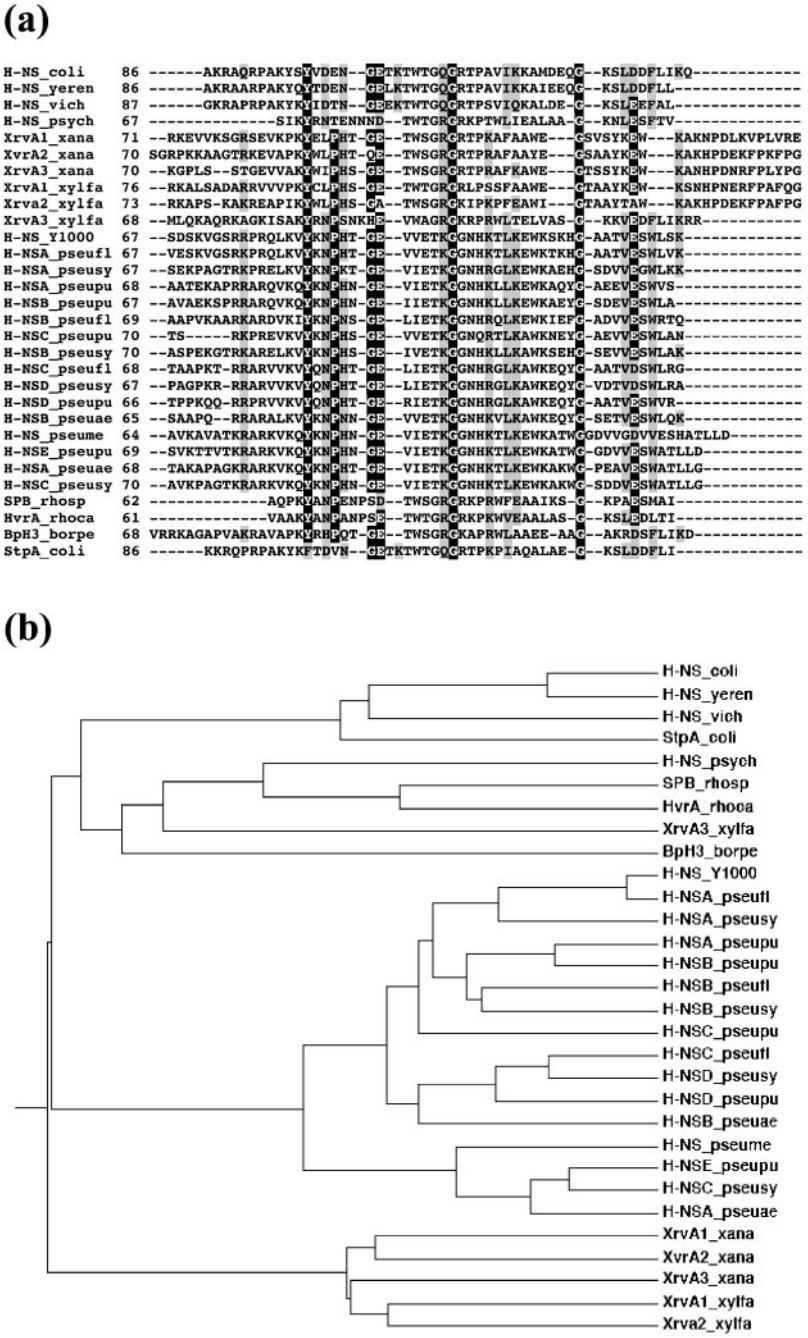

Fig. 1. MvaT proteins of *Pseudomonas* spp. and the H-NS family. Unfinished bacterial genome sequences were obtained from the Genomes OnLine Database (http://igweb.integratedgenomics.com/GOLD). H-NS-related sequences are from bacteria of the α-*Proteobacteria* (*Rhodobacter sphaeroides* and *Rhodobacter capsulatus*), β-*Proteobacteria* (*Bordetella pertussis*) and γ-*Proteobacteria* (*Escherichia coli*, *Yersinia enterocolitica*, *Vibrio cholerae*, *Psychrobacter* TAD1, *Xanthomonas axonopodis*, *Xylella fastidiosa*, *Pseudomonas* strain Y1000, *P. fluorescens*, *P. syringae*, *P. putida*, *P. aeruginosa* and *P. mevalonii*). (a) Structurally based alignment of H-NS-related proteins. The alignments were achieved using the program CLUSTAL W and refined manually as described previously (Bertin *et al.*, 1999, 2001; Tendeng *et al.*, 2003). Identical residues are in black boxes and similar residues are in grey boxes, with a threshold of 70 %. (b) Phylogenetic tree constructed by using a cluster algorithm from the GeneBee – Molecular Biology Server (http://www.genebee.msu.su).

such studies may lead to a better understanding of chromosome organization and the regulation of gene expression in bacterial cells.

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