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Serratia marcescens Contains a Heterodimeric HU Protein Like *Escherichia coli* and *Salmonella typhimurium*

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Homologs of the dimeric HU protein of *Escherichia coli* can be found in every prokaryotic organism that has been analyzed. In this work, we demonstrate that *Serratia marcescens* synthesizes two distinct HU subunits, like *E. coli* and *Salmonella typhimurium*, suggesting that the heterodimeric HU protein could be a common feature of enteric bacteria. A phylogenetic analysis of the HU-type proteins (HU and IHF) is presented, and a scheme for the origin of the *hup* genes and the onset of HU heterodimericity is suggested.

The HU protein was discovered 20 years ago (38) and has since been found in every gram-negative or -positive bacterium that has been investigated. It is also encoded in eukaryotic organelles (6, 46), in a virus (32), and in a bacteriophage (19). HU binds to DNA in a sequence-independent manner (3) but also recognizes specific structures in DNA (4, 37). HU is associated with the nucleoid, where it restrains DNA supercoiling and condenses the chromosome (40); its function can be compared to that of nucleosome-forming histones and HMG proteins in eukaryotic organisms (for a review, see reference 33). *Escherichia coli* HU is a small, basic, abundant, and predominantly heterodimeric protein composed of two 9-kDa subunits, HUα and HUβ (39). In the stationary phase, the major *E. coli* HU species is constituted by the HUαβ heterodimer (7). HU defines a new class of DNA-binding proteins (the HU-type family) sharing homologous DNA binding and dimerization domains. Interestingly IHF, which recognizes a specific DNA sequence (8), belongs to this class of proteins as well.

Cloning of the *hup* genes of *Serratia marcescens*. The heterodimeric nature of HU, confined so far to *Entererobacteriaceae* family members *E. coli* and *Salmonella typhimurium*, prompted us to investigate whether this feature could be found in other enteric bacteria. We therefore isolated chromosomal DNA from *S. marcescens* SM369 (a gift from Cécile Wandersman) by the method of Givskov et al. (18). The presence of *hup* genes in the genome of *S. marcescens*, which is slightly more GC rich than that of *E. coli*, was investigated by a PCR performed with *Taq* DNA polymerase as recommended by the supplier (Perkin-Elmer Cetus or Appligene) under the following conditions: 30 s at 94°C, 90 s at 52°C, and 90 s at 72°C for 30 cycles. A large collection of oligonucleotides designed to anneal at several locations upstream or downstream of the *E. coli hupA* and *hupB* open reading frames (ORFs) was exploited for this purpose. By using combinations of the upstream and downstream primers, all giving the expected amplified fragment with *E. coli* C600 DNA, it has been possible to identify pairs of oligonucleotides giving a band of the expected size upon amplification of *S. marcescens* DNA. The oligonucleotides used in this study were synthesized on the premises or purchased from Genset and are listed in Table 1. Remarkably, primers for both the *hupA* and *hupB* genes gave a positive signal, suggesting that the HU protein of *S. marcescens* is composed of two different polypeptides, as in *E. coli* and *S. typhimurium*. For both *hup* genes, a region corresponding to the ORF was amplified by using the oligonucleotide pairs HUPA-PCR1–HUPA-PCR2 and HUPB-PCR1–HUPB-PCR2. In each case, the 300-bp PCR products encompassing only the *hupA* and *hupB* ORFs were separated on 2% agarose–Tris acetate-EDTA gels, purified by using the Qiaex kit (Qiagen, Chatsworth, Calif.), digested with *Nde*I and *Bam*HI near their extremities, and cloned into *Nde*I-*Bam*HI-digested pJES307 (47) to yield, respectively, pT7HUPA-SM and pT7HUPB-SM. Longer *hupA* and *hupB* PCR products with extended 5′ ends were also obtained. With *hupA*, a PCR with oligonucleotides M1 and HUPA-PCR2 generated a 400-bp fragment which was isolated as described above, digested with *Bam*HI at the site carried by HUPA-PCR2, and cloned into *Sma*I-*Bam*HI-digested pJES307 to generate pHUPA-SM. A similar procedure was used with *hupB*, except that primers PB19 and HUPB-PCR2 were used to clone a 600-bp fragment into *Nru*I-*Bam*HI-digested pBR322 (2) to give plasmid pHUPB-SM. No amplification of *S. marcescens* sequences was obtained with primers designed to pair downstream of the ORF of either *E. coli hup* gene. To confirm the protein-coding capability of the cloned fragments, the *S. marcescens hupA* and *hupB* genes cloned downstream of a T7 promoter on plasmids pT7HUPA-SM and pT7HUPB-SM were overexpressed in *E. coli* BL21(DE3) (42). Upon isopropyl-β-D-thiogalactopyranoside (IPTG) induction of T7 RNA polymerase, strains BL21(DE3)pT7HUPA-SM and BL21(DE3) pT7HUPB-SM each accumulated, after 90 min, large amounts of polypeptides with calculated molecular masses of 9.5 kDa for HUα and 9.3 kDa for HUβ; these bands are absent in the extracts of uninduced cultures (data not shown). We have found that *E. coli* cells transformed with plasmids carrying either the *hupA* or *hupB* gene overproduce the relative HU subunit (36). This deregulation of HU synthesis dramatically induces the production of colanic acid, generating mucoid colonies. We used this phenotypic assay to test whether the cloned *S. marcescens hup* genes are functional in *E. coli*. All of the independent isolates of strain C600(pHUPB-SM) indeed produced mucoid colonies, whereas all isolates of strain C600 (pHUPA-SM) were normal (data not shown). The negative result for *hupA* could be explained by the absence of regulatory elements in the short segment upstream of the ORF on plasmid pHUPA-SM. DNA sequencing reactions were carried out as described by Sanger et al. (41) by using the four plasmids

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TABLE 1. Oligonucleotides used for PCR amplification of the *hupA* and *hupB* genes of *S. marcescens*

Gene	Oligonucleotide	Sequence[a]	Location[b]
hupA	M1	5′-**TGCGTGTATGCAGGAGAGTG**-3′	−84 to −65
	HUPA-PCR1	5′-GGAATTCCA**TATG**AACAAGACTCAACTG-3′ (*Nde*I)	−10 to +18
	HUPA-PCR2	5′-CGGGATCC**ACGCAATCTTACTTAACTGCG**-3′ (*Bam*HI)	+289 to +261[c]
hupB	PB19	5′-**GGCAGCGCATCGCGGCGGG**-3′	−391 to −373
	HUPB-PCR1	5′-GGAATTCCATA**TG**AATAAATCTCAATTGATCGAC-3′ (*Nde*I)	−10 to +24
	HUPB-PCR2	5′-CGGGATCC**GCTTAGTTTACCGCGTCTTTCAG**-3′ (*Bam*HI)	+283 to +253[c]

[a] The parts of the sequences in boldface are homologous to *E. coli* sequences.
[b] The locations indicated are relative to the first nucleotide of the *E. coli* ORFs.
[c] On the lower strand.

described above as double-stranded DNA templates. Both strands were sequenced by using T7 DNA polymerase (Pharmacia) and the oligonucleotides used for amplification. The resulting sequences of the *hupA* and *hupB* genes are shown in Fig. 1A and B, respectively. Translation of these sequences would generate, in both cases, a 90-amino-acid polypeptide, as is the case for *E. coli* and *S. typhimurium*. The calculated isoelectric points of *S. marcescens* HUα and HUβ are 9.57 and 10.27, respectively.

Sequence analysis and phylogenetic study. The protein and DNA databases contain up to now 38 protein sequences constituting the HU family (Table 2). These proteins were aligned globally with the PILEUP program of the University of Wisconsin Genetics Computer Group package (11), and phylogenic trees were generated with PILEUP and PHYLIP 3.5 (14). Since the output of both programs presented only minor differences, only the data produced by PILEUP are presented, for clarity. The phylogenic tree shown in Fig. 2 subdivides the HU-type proteins into four subfamilies. Subfamily I includes the site-specific IHF proteins, subfamily II contains the homodimeric HU proteins, subfamily III contains the heterodimeric HU proteins, and subfamily IV contains the more distant relatives of the family. Interestingly, the subdivision into subfamilies I, II, and III matches perfectly a classification

A

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   TATGCAGAAGTGCTGTCAATCGATATCCGTGCACTC  -75
GATGCTTTGCAAACGATAAACACACTGTAAGGATAACTT  -39
ATG AAC AAG ACT CAA CTG ATT GAT GTA ATC   30
 M   N   K   T   Q   L   I   D   V   I   10
GCG GAC AAG GCT GAC CTT TCC AAA GCA CAA   60
 A   D   K   A   D   L   S   K   A   Q   20
GCT AAA CTG GCT CTG GAA TCC ACC CTG GCT   90
 A   K   L   A   L   E   S   T   L   A   30
GCA ATT ACT GAG TCT CTG AAA GAA GGT GAT  120
 A   I   T   E   S   L   K   E   G   D   40
GCA GTA CAA TTG GTT GGC TTC GGT ACT TTC  150
 A   V   Q   L   V   G   F   G   T   F   50
AAA GTA AAC CAT CGT AGC GAG CGC ACT GGC  180
 K   V   N   H   R   S   E   R   T   G   60
CGC AAC CCA CAG ACT GGC AAA GAA ATC AAA  210
 R   N   P   Q   T   G   K   E   I   K   70
ATC GCA GCA GCC AAC GTG CCT GCG TTC GTT  240
 I   A   A   A   N   V   P   A   F   V   80
TCT GGC AAA GCA CTG AAA GAC GCA GTT AAG  270
 S   G   K   A   L   K   D   A   V   K   90
TAA GATTGCGTGGATCC                       287
 *
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B

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CGCTAAGTTAGATCTCTGTCGGCCCGCGTTTTGTCACCC  -78
AGTCGGTGGCTTGCAAGGTTCGATGGGATTGATATAACA  -39
GTG AAT AAG TCA CAA CTG ATC GAC AAG ATT   30
 M   N   K   S   Q   L   I   D   K   I   10
GCG GCA GGT GCT GAT ATT TCC AAA GCG GCA   60
 A   A   G   A   D   I   S   K   A   A   20
GCG GGA CGT GCT TTA GAC GCA GTA ATC GCT   90
 A   G   R   A   L   D   A   V   I   A   30
TCC GTT ACC GAC TCC CTG AAA GCA GGG GAT  120
 S   V   T   D   S   L   K   A   G   D   40
GAC GTG GCT CTG GTA GGT TTC GGT TCC TTT  150
 D   V   A   L   V   G   F   G   S   F   50
ACC GTG CGT GAA CGT TCG GCC CGT ACC GGC  180
 T   V   R   E   R   S   A   R   T   G   60
CGC AAC CCG CAG ACC GGT AAA GAG ATC AAG  210
 R   N   P   Q   T   G   K   E   I   K   70
ATC GCG GCA CGC AAA GTA CCT GCC TTC CGT  240
 I   A   A   R   K   V   P   A   F   R   80
GCA GGG AAA GCG CTG AAA GAC GCG GTA AAC  270
 A   G   K   A   L   K   D   A   V   N   90
TAA GCGGATCC                             281
 *
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FIG. 1. Nucleotide and deduced amino acid sequences of the *hupA* (A) and *hupB* (B) genes of *S. marcescens*. The asterisk indicates the stop codon.

TABLE 2. HU-type protein sequences analyzed in this study[a]

Locus	Accession code	Protein	Organism	Reference or source
CAJUHPB[b]	L25627	HUβ	*Campylobacter jejuni*	26
DBH1_RHILE	P02347	DNA-binding protein HRL18	*Rhizobium leguminosarum*	24
DBH5_RHILE	P02348	DNA-binding protein HRL53	*Rhizobium leguminosarum*	24
DBHA_ECOLI	P02342	HUα	*Escherichia coli*	23
DBHA_SALTY	P15148	HUα	*Salmonella typhimurium*	21
DBH_VIBPR	P28080	HU	*Vibrio proteolyticus*	17
DBHB_ECOLI	P02341	HUβ	*Escherichia coli*	22
DBHB_SALTY	P05515	HUβ	*Salmonella typhimurium*	29
DBH_ANASP	P05514	HU	*Anabaena* sp.	31
DBH_BACST	P02346	DNA-binding protein II (Hb)	*Bacillus stearothermophilus*	25
DBH_BACSU	P08821	DNA-binding protein II (Hb)	*Bacillus subtilis*	35
DBH_CLOPA	P05385	HU	*Clostridium pasteurianum*	43
DBH_CRYPH	P29214	HU	*Cryptomonas phi* chloroplast	46
DBH_THEAC	P02345	DNA-binding protein HTA	*Thermoplasma acidophilum*	9
DBH_THETH	P19436	DNA-binding protein II	*Thermus aquaticus*	48
DNZRHM	S00053	DNA-binding protein HRM	*Rhizobium meliloti*	27
HIU32727[b]	U32727	HU	*Haemophilus influenzae*	16
HIU32802[b]	U32802	IHFβ	*Haemophilus influenzae*	16
HIU32810[b]	U32810	IHFα	*Haemophilus influenzae*	16
IHFA_ECOLI	P06984	IHFα	*Escherichia coli*	30
IHFA_RHOCA	P30787	IHFα	*Rhodobacter capsulatus*	44
IHFA_SALTY	P15430	IHFα	*Salmonella typhimurium*	28
IHFA_SERMA	P23302	IHFα	*Serratia marcescens*	20
IHFB_ECOLI	P08756	IHFβ	*Escherichia coli*	15
IHFB_RHOCA	Q06607	IHFβ	*Rhodobacter capsulatus*	45
IHFB_SERMA	P23303	IHFβ	*Serratia marcescens*	20
L35044[c]	L35044	ORF99	*Mycoplasma gallisepticum*	A. Skamrov, unpublished data
MC352[b]	Z33259	HU	*Mycoplasma capricolum*	P. Bork, unpublished data
PSEHUPROTA[b]	L35257	HU	*Pseudomonas aeruginosa*	10
PSEIHFA[b]	L35258	IHFα	*Pseudomonas aeruginosa*	10
PSEIHFB[b]	L35259	IHFβ	*Pseudomonas aeruginosa*	10
S35616[c]	S35616	Hypothetical protein	African swine fever virus	32
S37140[c]	S37140	IHFα	*Erwinia chrysanthemi*	12
S37142[c]	S37142	IHFβ	*Erwinia chrysanthemi*	12
TF1_BPSP1	P04445	Transcription factor 1	Bacteriophage SPO1	19
TMOHDBP[b]	L23541	HU	*Thermotoga maritima*	P. G. Markiewicz, unpublished data
SMU25149	U25149	HUα	*Serratia marcesens*	This work
SMU25150	U25150	HUβ	*Serratia marcesens*	This work

[a] This work was based on the protein sequence entries from the PIR-SwissProt and GenBank databases (column 1). Column 2 contains the accession code for each sequence.

[b] When the amino acid sequence was not available, the deduced translation of the GenBank DNA sequence was used instead.

[c] The accession code was used as a sequence identifier in the absence of a locus name.

based on structural (homodimeric, heterodimeric) and functional (site-specific or non-site-specific DNA binding) criteria. Each subfamily can be further subdivided. Subfamilies Ia and Ib correspond, respectively, to the IHFβ and IHFα subunits of the α and γ proteobacteria. Subfamily IIa includes the HU proteins of the thermophilic bacteria *Thermus aquaticus* and *Thermotoga maritima*. Subfamily IIb contains the HU proteins of a blue-green alga (*Anabaena* sp.), of a eukaryotic chloroplast, and of *Clostridium pasteurianum*. The HU proteins from the α proteobacteria are classified in subfamily IIc, and the HU proteins of the *Bacillus* species are in subfamily IId. The HUα and HUβ subunits of the heterodimeric HU proteins are classified, respectively, in subfamilies IIIa and IIIb. To demonstrate the biological significance of this phylogenetic analysis, it is worth mentioning that antibodies raised against cyanobacterial HU cross-react more strongly with the HU-like protein from spinach chloroplasts than do anti-*E. coli* HU antibodies (6). This finding therefore confirms the validity of subfamily IIb, which contains the HU from *Anabaena* sp. and the HU from the chloroplast of the plant *Cryptomonas phi*. By sequence comparison of the cyanobacterial and *E. coli* HU proteins, Aitken and Rouviere-Yaniv (1) estimated the rate of evolution of HU to be on the order of 1% amino acid sequence difference per 5×10^7 years, a value comparable to that of histones H2A and H2B. This value makes HU a useful marker for measurement of evolution in prokaryotes.

Origin of the *hup* genes and heterodimeric HU. Even though proteins from subfamilies I (IHF) and III (HU) share over 30% identical residues, IHF cannot compensate for the absence of HU in the cell, as shown by Boubrik et al. (5). This observation prompted us to examine evolutionary links between the HU-type protein subfamilies to trace the appearance of the *hup* genes and to explain the origin of the heterodimeric nature of the HU proteins of enteric bacteria. The phylogenetic tree of the HU-type proteins in Fig. 2 indicates that the integration host factor IHFα and IHFβ subunits (subfamily I) arose from a common ancestor in very remote times, probably by gene duplication. Subfamily II of HU proteins seems to have derived more recently from the same ancestral protein that gave rise to the IHF subunits. This group contains the homodimeric HU species from which subfamily III (HUα and HUβ subunits) later evolved. Interestingly, Drlica and Rouviere-Yaniv (13) had made the same hypothesis by direct comparison of the amino acid sequences of *E. coli* IHFα, IHFβ,

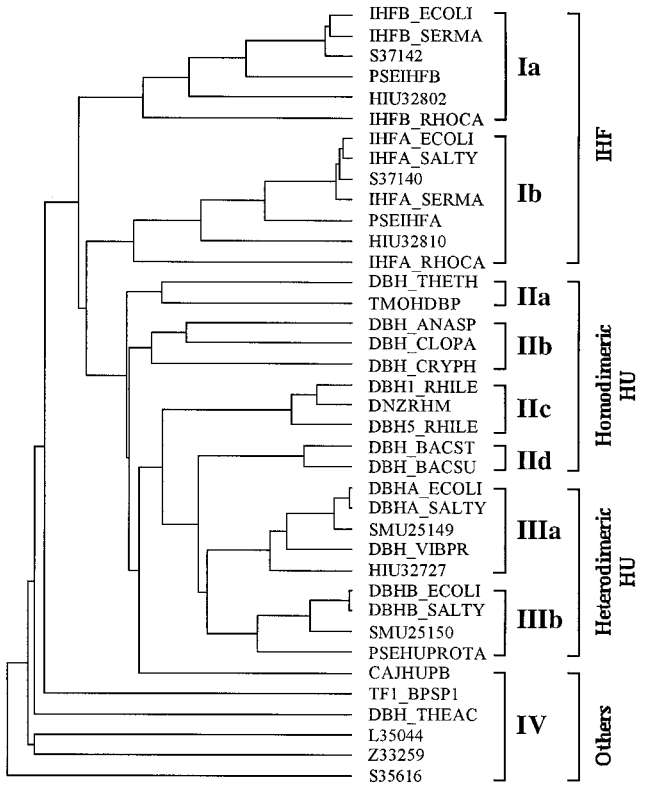

FIG. 2. Phylogenetic tree generated by PILEUP on the basis of global alignment of the 38 sequences listed in Table 2 (the alignment is not shown). The GenBank or PIR-SwissProt code for each sequence is given. The subdivision of the HU-type proteins into subfamilies is indicated on the right.

A

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      1                                30
E.c.  MNKTQLIDVIAEKAELSKTQAKAALESTLAAITESLKEGDAVQLV
S.t.  ..........D.............................
S.m.  ..........D..D...A...L.....................
V.p.  ........F.....D...A.......A..DGV.DA.....Q...I
H.i.  ....D...A..NA...N.K.......A..D...A.....EP...I

                  60                              90
E.c.  GFGTFKVNHRAERTGRNPQTGKEIKIAAANVPAFVSGKALKDAVK
S.t.  ..........................................
S.m.  ..........S...............................
V.p.  ..........A......K..A..Q..........A.........
H.i.  ........E..A.........A..Q...SK.............I.
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B

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      1                                30
E.c.  MNKSQLIDKIAAGADISKAAAGRALDAIIASVTESLKEGDDVALV
S.t.  .......E..................................
S.m.  ............................V.....D...A.......
P.a.  ....E...A...S...P.DV.......V.E...GA..A..S.V..

                  60                              90
E.c.  GFGTFAVKERAARTGRNPQTGKEITIAAAKVPSFRAGKALKDAVN
S.t.  ..........................................
S.m.  ...S.T.R..S.............K...R...A............
P.a.  .........P............P.K...-.I.G.K..........
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FIG. 3. Global alignment of the HUα and HUβ subunits. (A) Global alignment of the HUα and related proteins from *V. proteolyticus* (V.p.), *H. influenzae* (H.i.), and the enteric bacteria *E. coli* (E.c.), *S. typhimurium* (S.t.), and *S. marcescens* (S.m.). (B) Global alignment of the HUβ and related proteins from *P. aeruginosa* (P.a.) and the enteric bacteria *E. coli* (E.c.), *S. typhimurium* (S.t.), and *S. marcescens* (S.m.). Only the residues that differ from the *E. coli* sequences are shown; dots and hyphens indicate conserved residues and gaps, respectively.

HUα, and HUβ. The multiple alignments relative to families IIIa and IIIb, presented in detail in Fig. 3A and B, comprise the *S. marcescens* HU proteins and those of the other heterodimeric members of the family *Enterobacteriaceae*, *E. coli* and *S. typhimurium*. *Vibrio proteolyticus*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* do not belong to the enteric bacteria and encode only a homodimeric HU species. However, their sequences were included in this comparison because of their high similarity to the enteric HUα or HUβ sequences. Another striking common property of this set of bacteria is that the *hupB* ORF for each of the four species listed in Fig. 3B starts with a GUG codon. On the evolutionary scale, the appearance of the heterodimeric nature of HU can be explained by two models. The first model involves duplication of the *hup* genes in some bacterial ancestor, as occurred for the IHF-encoding genes but in much more recent times. The *hup* genes have then evolved to code for present-day heterodimeric HU. Three findings suggest an alternative model for the origin of heterodimericity. First, the group of Oppenheim, studying the HU protein of *V. proteolyticus*, confirmed the presence of a single *hupA*-like gene in that organism by Southern analysis (17). Second, the complete sequence of the *H. influenzae* genome (16) revealed the existence in that organism also of a unique *hupA*-like gene. Third, the amino-terminal sequence of HU isolated from *P. aeruginosa* shows that this protein is an HUβ2-like homodimer (10). Our phylogenetic analysis has assigned these homodimeric proteins to subfamilies IIIa and IIIb, containing the α and β subunits of the heterodimeric HU species. Phylogenetic studies based on 16S rRNA sequences classify bacteria similar to *V. proteolyticus*, *P. aeruginosa*, and *H. influenzae* as being the direct ancestors of the enteric bacteria (34). We can therefore consider a second model for the origin of the heterodimeric HU implying horizontal transfer of an already well-diversified *hupB* gene into a host carrying a single *hupA* gene, as in the three species listed above, to yield an organism capable of synthesizing a HUαβ heterodimer. Interspecies transfer of genes between *E. coli* and other enteric bacteria has been observed, as discussed by Ochman and Wilson (34). This work has demonstrated that the HU protein of *S. marcescens* is probably heterodimeric in nature, as in *E. coli* and *S. typhimurium* and, potentially, other enteric bacteria. This assumption is further confirmed by the presence of the genes *hupA* and *hupB* in *Erwinia chrysanthemi* (13a). Analysis of a larger pool of *hup* genes will probably demonstrate a specific role of the heterodimeric HU protein and explain its confinement by evolution to defined bacterial groups.

Nucleotide sequence accession numbers. The nucleotide sequences of the *S. marcescens hupA* and *hupB* genes have been submitted to GenBank and assigned accession numbers U25149 and U25150.

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