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Histone-like protein of *Streptomyces lividans*

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A DNA-binding protein (about 10 kDa and pI>9.7) of *Streptomyces lividans* TK24 was purified on a denatured DNA-Cellulose column, and then on a native DNA-Cellulose column. The N-terminal amino acid sequence of this protein had high homology with those of small basic DNA-binding proteins known as histone-like proteins. Thus, this protein was designated HSl (histone-like protein of *S. lividans*). Gel retardation assay revealed that HSl bound with the single-stranded DNA as replication intermediates of pSA1.1. We propose that HSl may participated in the replication of pSA1.1.

The *hup* gene encoding HSl was cloned and sequenced. The deduced N-terminal amino acid sequence, molecular mass (9851 Da) and pI (9.95) were in good agreement with characteristics of HSl. HSl had the signature sequence for the histone-like proteins. Phylogenetic analysis suggested that HSl did not belong to the cluster of histone-like proteins from most of bacteria. The *hup* transcript of about 500 nucleotides was detected. The *hup* fragment hybridized with the *Ase*I fragment C in the 9–10 o'clock region of the chromosome. Total DNAs of many *Streptomyces* species hybridized with the internal region of *hup*.

INTRODUCTION

Streptomyces strains have linear chromosomes of about 8000-kb (Lin *et al.*, 1993) and develop vegetative mycelia to aerial mycelia, in which their cells are multinucleoidal, and finally to uninucleoidal spores. Little is known of factors involved in the maintenance of chromosome structure, DNA replication, partitioning and segregation.

Histone-like proteins are small basic DNA-binding proteins (Drlica and Rouviere-Yaniv, 1987). The bacterial histone-like protein HU, also known as DNA chaperone (Travers *et al.*, 1994) has a role in several DNA-protein interactions such as formation of the nucleosome-like structure and DNA replication reviewed in references (Drlica and Rouviere-Yaniv, 1987; Oberto *et al.*, 1994).

This report deals with characterization of the histone-like protein HSl of *S. lividans* TK24 and cloning of the *hup* gene encoding HSl.

MATERIALS AND METHODS

Bacterial strains, plasmid and media

Streptomyces lividans TK24, *S. lividans* ZX7 and *S. coelicolor* M145 (Hopwood *et al.*, 1985) were kindly provided by D. A. Hopwood, K. F. Chater and T. Kieser. Other *Streptomyces* species were purchased from the American Type Culture Collection

(ATCC) and the Japan Collection of Microorganisms (JCM). The plasmid pSA1.1 was originally isolated from a derivative strain, PK100, of *S. azureus* ATCC14921 (Miyoshi *et al.*, 1986). *Streptomyces* strains were grown at 28 °C in YEME medium (Hopwood *et al.*, 1985) supplemented with 0.5% glycine.

Buffers

Buffer A was 20 mM Tris-HCl (pH 7.4), 1 mM 2-mercaptoethanol, 50 mM NaCl, 1 mM EDTA and 10% glycerol. Buffer B was 20 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2 \cdot 6H_2O$, 2 mM $CaCl_2 \cdot 2H_2O$, 1 mM EDTA and 1 mM 2-mercaptoethanol. Buffer C had the same composition of buffer A without glycerol.

1xTAE buffer consisted of 40 mM Tris-acetate (pH 8.0) and 1 mM EDTA. 0.5xTBE buffer consisted of 45 mM Tris-borate (pH 8.0) and 1 mM EDTA. 1xMOPS buffer consisted of 20 mM 3-(*N*-morpholino) propanesulphonic acid (pH 7.0), 5 mM sodium acetate and 1 mM EDTA.

Protein isolation and manipulation

DNA-free extract was prepared using the following procedures. The mycelia from liquid culture were washed twice in buffer B and suspended in buffer B. The mycelia were sonicated on ice. After centrifugation the supernatant was treated with 20 μg ml^{-1} DNase I for 30 min at 25 °C, adjusted to 1.7 M NaCl and 10% polyethylene glycol 6000 and kept for 30 min at 0 °C. After centrifugation the supernatant was dialysed against buffer C, and then centrifuged to remove any precipitate, glycerol was added to the supernatant to a final concentration of 10%.

Single-stranded (ss) DNA- and double-stranded (ds) DNA-binding proteins were prepared by affinity chromatography on denatured and native DNA-Cellulose columns (Pharmacia Biotech), respectively. The DNA-binding proteins were eluted stepwise with buffer A containing 0.1, 0.2, 0.3, 0.4, 0.5 and 2 M NaCl.

Two dimensional-polyacrylamide gel electrophoresis (2D-PAGE) was done by isoelectronic focusing, using the SJ-1060 DCII apparatus (Atto, Tokyo, Japan) to be followed by 20% SDS-PAGE. The gel was then stained with Coomassie brilliant blue R-250.

The N-terminal amino acid (aa) sequence of protein was determined using a gas-phase sequencer, PSQ-1 (Shimadzu, Kyoto, Japan) and System 890M/E Sequencer (Beckman).

DNA isolation and manipulation

Total DNA was prepared according to the method of te Riele *et al.* (1986). Conventional procedures were used for manipulations of DNA (Hopwood *et al.*, 1985; Sambrook *et al.*, 1989).

Southern hybridization was performed using DIG DNA Labeling and Detection kit (Boehringer Mannheim).

For the gel retardation assay, DNA and protein mixture in buffer A was electrophoresed on a 0.7% agarose gel in 1xTAE buffer. The DNA-protein complexes were then detected by Southern hybridization.

The nucleotide sequence was determined on both strands by the dideoxy chain-

termination method using a Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham).

The chromosomal DNA in agarose plug was prepared according to the method of Leblond *et al.* (1993). Pulsed-field gel electrophoresis (PFGE) was performed on 1% agarose gel in 0.5xTBE buffer, pulse times from 50 to 130 sec, 6 V/cm for 24 hr.

RNA isolation and manipulation

Total RNA of *S. lividans* TK24 was prepared using the RNeasy Total RNA Kit (Qiagen) and electrophoresed on 1.2% agarose-2.2 M formaldehyde gel in 1xMOPS buffer. Northern hybridization was performed using DIG DNA Labeling and Detection kit (Boehringer Mannheim).

Polymerase chain reaction (PCR)

The reaction mixture for PCR contained 10 mM Tris-HCl (pH9.0), 50 mM KCl, 0.1% Triton X-100, 7% dimethylsulfoxide, 2 mM $MgCl_2 \cdot 6H_2O$, 200 μM of each of the four dNTPs, 2.5U of *Taq* polymerase, 50 pmol of each primer and 100 ng of chromosomal DNA in a final volume of 100 μl. After denaturation at 95 °C for 5 min, amplification was performed with the following steps, denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min and polymerization at 72 °C for 1 min by 30 cycles.

Computer analysis of amino acid and nucleotide sequences

Comparison of aa sequence with PIR and PRF databases was performed using the BLAST network service (Altschul *et al.*, 1990). The aa sequence motif was searched using PROSITE (Bairoch, 1992). Phylogenetic analysis was performed by the UPGMA method (National Institute of Genetics, Mishima, Japan) on the basis of alignment generated by PILEUP (Devereux *et al.*, 1984).

RESULTS AND DISCUSSION

Purification of the histone-like protein HSl

A DNA-binding protein was purified from the DNA-free extract of *S. lividans* TK24 on a denatured DNA-Cellulose column, then on a native DNA-Cellulose column. This protein was eluted with buffer A containing 0.3 M and 0.2 M NaCl from denatured and native DNA-Cellulose columns, respectively. Thus this protein had a higher affinity to ssDNA than to dsDNA. 2D-PAGE analysis revealed that this protein was focused at more than pI 9.7 and had a molecular mass of 10 kDa (Fig. 1). The N-terminal aa sequence of this small basic DNA-binding protein was determined as MNRSELVAALADRAE. This aa sequence showed a high homology with those of small basic DNA-binding proteins called bacterial histone-like proteins, (identity, similarity); HRm from *Rhizobium meliloti* (Laine *et al.*, 1983), (71%, 100%); HAt from *Agrobacterium tumefaciens* (Khanaka *et al.*, 1985), (57%, 100%); HU-2 from *Escherichia coli* (Kano *et al.*, 1987), (40%, 86%) and others. Thus, this protein was designated HSl (histone-like protein of *S. lividans*), according to the nomenclature system of Drlica and Rouviere-Yaniv (1987). HSl had a higher affinity to ssDNA than to dsDNA, in agreement with the property of HU protein in *E. coli* (Holck and Kleppe, 1985).

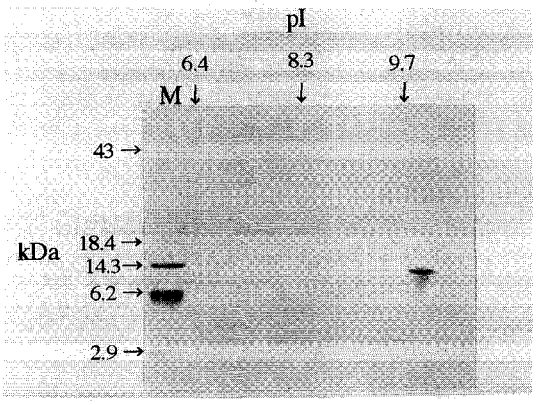

Fig. 1 2D-PAGE analysis of the purified DNA-binding protein. Lane M, protein molecular weight standards (Gibco BRL)

Gel retardation assay of pSA1.1 by HSI

HSI was analysed by gel retardation assay (Fig. 2). pSA1.1 replicates by a rolling-circle mechanism, so accumulates ssDNA as replication intermediates. When the amount of HSI was increased, migration of the ssDNA band of pSA1.1 was retarded. This shows that HSI binds with ssDNA as the replication intermediates of pSA1.1. We thus assume

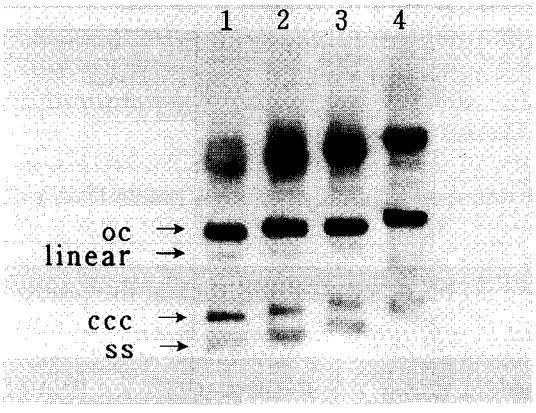

Fig. 2 Gel retardation assay of pSA1.1 with HSI protein. The total DNA of *S. lividans* TK24 harboring pSA1.1 (10 μg) was electrophoresed on a 0.7% agarose gel with various amounts of HSI. And then Southern hybridization was performed using pSA1.1 DNA as a probe. Lane 1, no protein; lane 2, 60 ng; lane 3, 150 ng; lane 4, 300 ng.

that HSl probably participates in the replication of pSA1.1. Histone-like proteins are known to be involved in theta replications of chromosome (Yung and Kornberg, 1989) and plasmid (Ogura *et al.*, 1990), but we found no reports regarding the rolling-circle replication. Further investigation such as gene disruption will demonstrate the function of HSl in the replication of pSA1.1.

Amplification of the internal region of hup

To amplify the internal sequence of *hup* using PCR, based on the N-terminal aa sequence of HSl and the C-terminal aa sequence of HRm, two oligonucleotides, 5'-GAGCTGGTCGCCGCCCTGGC for forward primer and 5'-GTTGACGGCGTCCTTCAGGCC for reverse primer were synthesized according to the specific codon usage of *Streptomyces* (Wada *et al.*, 1992). Several non-specific fragments were amplified in PCR. The PCR products were then separated by 1.5% agarose gel electrophoresis. About 300 bp fragments were extracted from the agarose gel and served as templates in the nested PCR. Two oligonucleotides, 5'-GCCCTGGCCGACCG(G/C)GCCGA for forward primer and 5'-CAGGCCCTTGCCGGCGGTGA for reverse primer were synthesized. The nested PCR was performed under the same reaction conditions as the first PCR, except for the template and primers.

Cloning of the hup gene encoding HSl

The nested PCR product was used as the probe to screen *hup* from the gene library of *S. lividans* TK24. A positive clone carrying a recombinant plasmid which contained a 2.3-kb *Hinc* II fragment was obtained. The internal 480 bp sequence of the fragment was determined (Fig. 3). This nucleotide sequence revealed the putative *hup* encoding HSl.

```
AATGTCAGAGGGCCCTGGCAGCATCGGCATCGCTGTGCCCAACAATGCGGCTCCGCCGCG    60

TGGCACGCCTCAACGGCAAGAAGAACACGGGAGTAACAACATGAACCGCAGTGAGCTGGT   120
                                    M  N  R  S  E  L  V          7

GGCCGCGCTGGCCGACCGCGCCGAGGTGACCCGCAAGGACGCCGACGCCGTGCTGGCCGC   180
 A  A  L  A  D  R  A  E  V  T  R  K  D  A  D  A  V  L  A  A     27

CTTCGCCGAGGTTGTCGGCGACATCGTCTCCAAGGGCGACGAGAAGGTCACCATCCCCGG   240
 F  A  E  V  V  G  D  I  V  S  K  G  D  E  K  V  T  I  P  G     47

CTTCCTGACTTTCGAGCGCACCCACCGTGCCGCTCGCACCGCCCGCAACCCGCAGACCGG   300
 F  L  T  F  E  R  T  H  R  A  A  R  T  A  R  N  P  Q  T  G     67

CGAGCCGATCCAGATTCCGGCCGGCTACAGCGTCAAGGTCTCCGCGGGCTCCAAGCTCAA   360
 E  P  I  Q  I  P  A  G  Y  S  V  K  V  S  A  G  S  K  L  K     87

GGAAGCCGCCAAGGGCAAGTAGGCGCTCCGCTTGGAGTGCCGGGGACTGCGCGGATCGTT   420
 E  A  A  K  G  K  *                                            93

CGTTCGGGACGCGGTCGTTTCGCGGCTGCGGGTCGTTCGTGGCTGGTCGCGCAGTTCCCC   480
```

Fig. 3 Nucleotide sequence of the coding and flanking regions of *hup*. The deduced aa sequence is given below the nucleotide sequence in single letter code. The asterisk indicates the stop codon. This nucleotide sequence is deposited in the DDBJ, EMBL and GenBank databases with the accession number AB001381.

Table. 1 Comparison of the deduced amino acid sequence of HSI with those of other histone-like proteins.

Protein, Organism	identity (%)
HU, *Anabaena*	50.0
DNA-binding protein II, *Clostridium*	44.3
HU, *Vibrio*	42.9
HU, *Pseudomonas*	42.9
HU, *Haemophilus*	42.9
HBsu, *Bacillus*	40.9
HU, *Thermotoga*	40.7
TF1, phage SPO1	40.2
HU-2, *Salmonella*	39.6
HU-1, *Salmonella*	39.6
HU-1, *Escherichia*	39.6

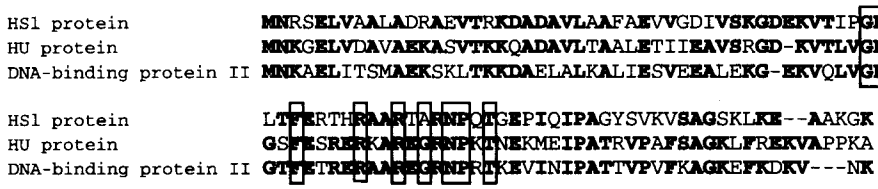

Fig. 4 Comparison of the aa sequences of HSl (*Streptomyces*), HU (*Anabaena*) (Nagaraja and Haselkorn, 1994) and DNA-binding protein II (*Clostridium*) (Kimura *et al.*, 1984). Identical aa residues are bold-faced. Hyphens (-) represent gaps in the alignment. The conserved residues of the signature sequence for HU-type histone-like proteins are boxed.

The deduced N-terminal aa sequence, molecular mass (9851 Da) and pI (9.95) are in good agreement with characteristics of HSl.

Comparisons of the deduced aa sequence of HSl with the PIR and PRF databases revealed a high degree of homology to those of various histone-like HU-type proteins (Table 1, Fig. 4). The complete deduced aa sequence of HSl has a relatively lower homology to that of HRm (identity, 37.4%), the N-terminal aa sequence of which has the highest homology with that of HSl.

The aa sequence motif was searched in the deduced aa sequence of HSl. HSl had the signature sequence for the HU-type histone-like proteins, [GSK]-F-x(2)-[LIVMF]-x(4)-[RKEQA]-x(2)-[RST]-x-[GA]-x-[KN]-P-x-T (Fig. 4). This aa sequence pattern spans the first half of the flexible DNA-binding arm of histone-like proteins.

Phylogenetic analysis of HSl

Phylogenetic analysis of twenty six histone-like proteins was performed (Fig. 5). Most of HU-type histone-like proteins were grouped into the B cluster. But HSl belonged to A cluster. TF1 is the histone-like protein of the linear double-stranded DNA phage SPO1 of *Bacillus* (Greene *et al.*, 1984). *Streptomyces* has a linear double-stranded

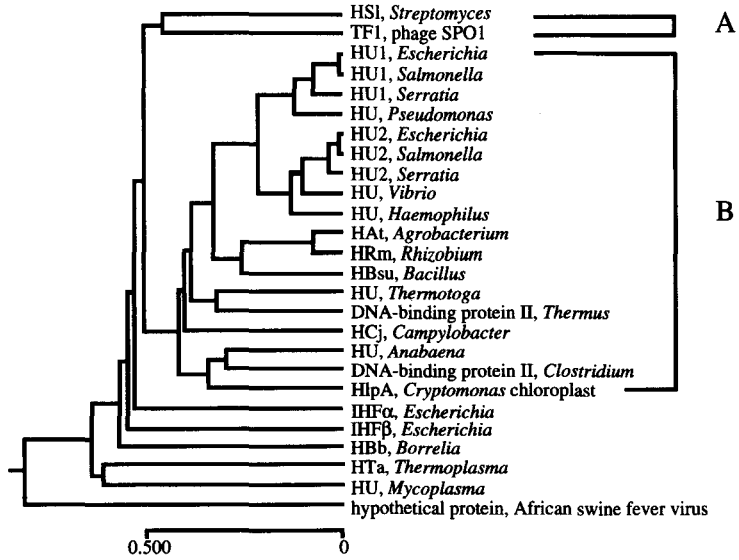

Fig. 5 Phylogenetic analysis of twenty six histone-like proteins. The scale bar indicates evolutionary distance.

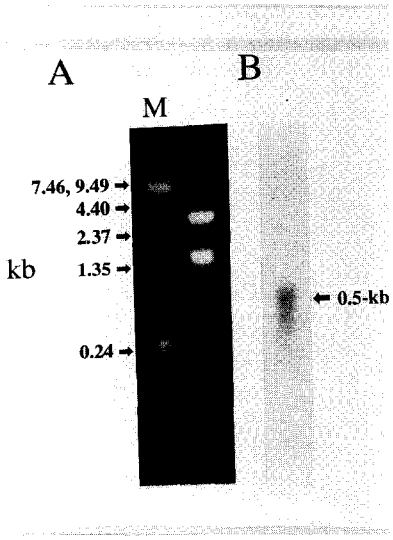

Fig. 6 Detection of the *hup* transcript. Panel A is the electrophoresis profile of total RNA of *S. lividans* TK24. Lane M, 0.24–9.5 Kb RNA Ladder (Gibco BRL). Panel B is Northern hybridization analysis using the internal sequence of *hup* as a probe

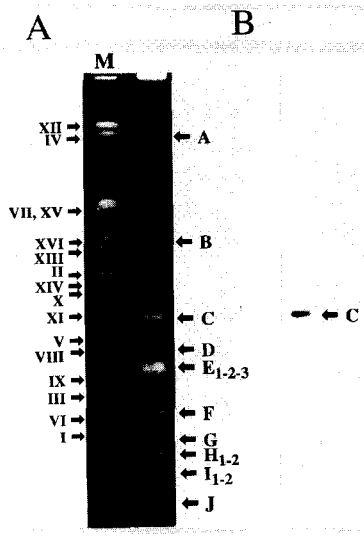

Fig. 7 Physical mapping of *hup*. Panel A is PFGE analysis of *Ase* I -digested chromosomal DNA of *S. lividans* ZX7. Lane M, Yeast DNA-PFGE Markers (Pharmacia Biotech). The restriction fragments are designated in accord with the nomenclature system of Leblond *et al.* (1993). Panel B is Southern hybridization analysis using the *hup* fragment as a probe.

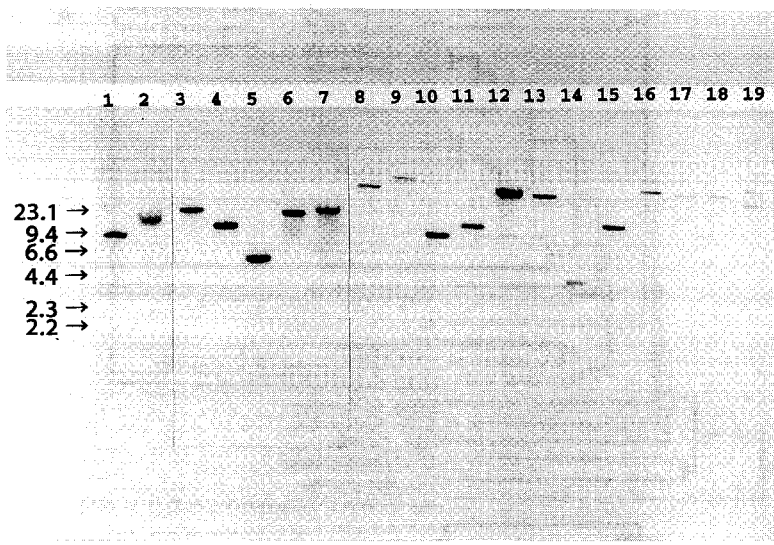

Fig. 8 *Streptomyces* species possessing a *hup* homolog. *Bam*HI-digested total DNAs were electrophoresed on 0.7% agarose gel, and then Southern hybridization analysis was performed using the internal sequence of *hup* as a probe. Lane 1, *S. achromogenes* JCM4121; lane 2, *S. albidoflavus* JCM4446; lane 3, *S. albus* JCM4005; lane 4, *S. antibioticus* JCM4007; lane 5, *S. azureus* ATCC14921; lane 6, *S. celluloflavus* JCM4126; lane 7, S. *coelicolor* M145; lane 8, *S. coeruleorubidus* JCM 4359; lane 9, *S. coerulescens* JCM4360; lane 10, *S. cyaneus* JCM4220; lane 11, *S. fradiae* JCM4133; lane 12, *S. glaucescens* JCM4377; lane 13, *S. griseoluteus* JCM4041; lane 14, *S. griseus* JCM4046; lane 15, *S. hawaiiensis* JCM4172; lane 16, *S. laurentii* ATCC31255; lane 17, *S. lavendulae* JCM4055; lane 18, *S. lividans* TK24; lane 19, *S. viridochromogenes* JCM4856.

chromosome, too. So it was supposed that the similar DNA structure leaded to the same phylogenetic cluster in histone-like protein. Whereas *Borrelia* also has a linear chromosome (Casjens and Huang, 1993), HBb (Tilly *et al.*, 1996) did not belong to A cluster. This contradiction may be based on the terminal structure of its chromosome. The 5' termini of *Streptomyces* chromosome are bound to proteins (Lin *et al.*, 1993), however *Borrelia* chromosome termini are supposed to have hairpin structures (Tilly *et al.*, 1996).

Northern hybridization analysis of hup transcript

Northern hybridization analysis was performed using the internal sequence of *hup* as a probe (Fig. 6). As a transcript of about 500 nucleotides was detected, *hup* (282 nucleotides) may be transcribed into a monocistronic mRNA.

Physical mapping of hup

The physical map of the chromosome of *S. lividans* ZX7 was constructed by Leblond *et al.* (1993). Both *S. lividans* TK24 and *S. lividans* ZX7 are derivatives of *S. lividans* 66. The chromosomal DNA was digested by *Ase*I and separated by PFGE, followed by

Southern hybridization analysis using the 2.3-kb *Hinc* II fragment containing *hup* as a probe (Fig. 7). The probe hybridized with the *Ase* I fragment C in the 9–10 o'clock region of the chromosome. Thus, *hup* locates on the 9–10 o'clock region.

Wide distribution of hup in Streptomyces

Total DNAs were prepared from the nineteen *Streptomyces* species. Southern hybridization analysis was performed using the internal sequence of *hup* as a probe (Fig. 8). As the total DNAs of all tested nineteen *Streptomyces* species hybridized with the probe, the *hup* homolog is widely distributed to *Streptomyces* species.

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