Cloning and Characterization of the *recA* Gene of *Aquaspirillum magnetotacticum**

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Abstract

The *recA* gene of *Aquaspirillum magnetotacticum* has been isolated from a genomic library and introduced into a *recA* mutant strain of *Escherichia coli* K12. The cloned gene complemented both the recombination and DNA repair deficiency of the host and its protein product promoted the proteolytic cleavage of the LexA protein. A protein whose molecular weight is similar to that of the RecA protein of *E. coli* was associated with the cloned sequence.

Key words: *recA* gene - *Aquaspirillum magnetotacticum* - gene library - recombinant cosmid

Aquaspirillum magnetotacticum is a gram-negative freshwater spirillum that synthesizes nanometer-sized, single-domain magnetic particles (for review see Blakemore 1982). We have recently constructed a gene library from the genomic material of this organism, which we have used to complement auxotrophic mutant strains of *E. coli* K12 (Waleh 1988). To investigate whether mutations other than amino acid auxotrophy could be complemented with *A. magnetotacticum* genes, we screened the library for sequences that would complement the *recA* function of *E. coli* K12.

RecA-like sequences have been isolated from a number of bacterial species (West et al. 1983; Pierre and Paoletti 1983; Keener et al. 1984; Ohman et al. 1985; Goldberg and Mekalanos 1986; Koomey and Falkow 1987). The *recA* gene product in *E. coli* is involved in homologous recombination (Clark 1973) and DNA repair (Hanawalt et al. 1979; Walker 1984). This protein also regulates the expression of a number of unlinked chromosomal genes by promoting the proteolytic cleavage of their repressor molecule, the LexA protein (Walker 1984). The LexA protein is also the the repressor of the *recA* gene (Mount 1977).

In this paper, we report the cloning and characterization of the *recA* gene of *A. magnetotacticum*. Hybridization experiments indicate that homology exists between the *recA* sequences of *E. coli* and *A. magnetotacticum*, and complementation studies suggest that the two RecA proteins are functionally similar. A protein whose gel migration pattern is similar to the RecA protein of *E. coli* is produced when recombinant clones are treated with DNA-damaging agents.

Materials and Methods

Bacteria and plasmids. *A. magnetotacticum* strain MS-1 was provided by BioMagnetech Corporation. Strain HB101, F^- *hsd20* (rB^- mB^-) *recA13 ara-14 proA2 leuB6 thi-1 lacY1 galK2 xyl1 mtl1 supE44 str* λ, was used to propagate the gene library. CL142 (K12-Row) was used as the colicin indicator strain (Ozeki et al. 1962). Plasmid pJC859 (provided by John Clark, University of California at Berkeley, Berkeley, California, USA) is a pBR322 derivative and carries the *E. coli recA* gene.

Culture conditions. *A. magnetotacticum* strain MS-1 was grown according to the procedures described by Blakemore et al. (1979). *E. coli* cells were grown in LB liquid or LB agar medium. For the induction of RecA protein, cells were grown in M9 medium supplemented with 0.3% casamino acids and 0.2% thiamine. Ampicillin (amp) was added at the final concentration of 50 μg/ml when required. MMS plates were prepared by spreading 200 μl of a 2% aqueous solution on the surface of LB plates.

Cloning the recA gene. A gene library from DNA of *A. magnetotacticum* was constructed in a broad host-range cosmid c2RB (Bates and Swift 1983) as described previously (Waleh 1988).

Purification of plasmids and DNA fragments. Plasmids were purified by the procedure of Rodriguez and Tait (1983). DNA fragments were purified from low-percentage SEAPLAQUE gels (FMC Corporation, Rockland, ME).

UV survival measurements. Cells were grown in LB-amp liquid medium at 37°C overnight. They were then pelleted by centrifugation and resuspended in an equal volume of TEN (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 150 mM NaCl) buffer. The cell suspension was serially diluted in the above buffer and 0.01 ml volumes of dilutions were spotted on LB agar-amp medium. Plates were placed at a distance of 82 cm from a 15-watt germicidal low-pressure mercury lamp (GE G8T5) and irradiated for the indicated time periods. Plates were wrapped in aluminum foil to prevent photo-reactivation and incubated at 37°C overnight. The cross-streak method was used for screening a large number of colonies. In this test, overnight-grown cultures were streaked across LB agar-amp plates, and one-half of each streak was irradiated for 30 sec. Plates were incubated at 37°C overnight in the dark. The RecA$^-$ mutant cells were killed at this UV dose, and the RecA$^+$ cells produced a thin film of growth in the irradiated parts of the streak.

Southern blot analysis. Plasmids were digested with *Eco*RI, electrophoresed in a 0.8% agarose gel, denatured *in situ*, and transferred to nitrocellulose filters as described by Maniatis et al. (1982). *Eco*RI-digested DNA fragments of recombinant cosmids were labeled with [γ^{32}P]ATP by T4 DNA kinase and were used as probe. The probe from *E. coli recA* gene was prepared by nick-translation using a BRL kit. The nitrocellulose filters were baked in an 80°C vacuum oven for 2 h and hybridized for 22 to 24 h at 45°C in a solution containing 50% formamide, 5xSSC (0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0), 0.8% Denhardt's solution (Maniatis et al. 1982), and 300 µg of heat denatured salmon sperm DNA. Filters were washed at

room temperature in 2xSSC-0.1% sodium dodecyl sulfate (SDS) for 20 min, at 45°C in 0.2xSSC-0.1% SDS for 30 min, wrapped in Saran wrap, and exposed to X-ray film at -70°C using an intensifying screen (Cronex Hi-Plus).

Colicin test. Colonies were spotted on LB agar plates and were incubated at 37°C. After overnight incubation, cells were killed by exposure to chloroform vapor for 30 min and overlayed with 3 ml soft agar seeded with strain CL142. The colicin-producing colonies produced a zone of inhibition in the lawn of the indicator strain.

Induction of RecA protein. Cells were grown in minimal medium in a shaking 37°C incubator to an optical density of about 0.5-0.6 at 660 nm. At this time, cells were exposed either to UV light for the indicated periods of time or to mitomycin C added to the cultures at the final concentration of 1 μg/ml. Cells were shaken at 37°C in the dark for two additional hours. Samples were taken at indicated times; cells were pelleted and stored at -20°C.

Polyacrylamide gel electrophoresis. Cell pellets were resuspended in a dye mixture consisting of 1% SDS, 20% glycerol, 40 mM Tris-HCl, pH 6.8, 0.05% bromphenol blue (BPB), and 0.14 M 2-mercaptoethanol, boiled for 5 min in a boiling water bath, and electrophoresed through a 15% acrylamide gel. Cells were stained with Coomassie brilliant blue.

Results

Isolation of the *A. magnetotacticum recA* gene. The gene library prepared from chromosomal DNA of *A. magnetotacticum* was propagated in HB101, a *recA* mutant strain of *E. coli* K12. This strain is sensitive to DNA-damaging agents, such as MMS, because of its deficiency in homologous recombination and DNA repair functions. Library clones that grow in the presence of MMS should therefore carry sequences that complement the *recA* deficiency and allow the growth of their host strain. Of 542 amp-resistant (amp^r) clones tested, we found 5 that grew in the presence of MMS. Plasmid analysis indicated that all clones carried recombinant cosmids with inserts that were between 26-35 kb in size. The *EcoRI* digestion patterns of the recombinant cosmids is shown in Fig. 1A. *EcoRI* digestion separates the insert DNA from the vector DNA. Southern hybridization analysis of the *EcoRI* digests of the recombinant cosmids using one of the $RecA^+$ clones as probe (Fig. 1B) demonstrates that all five $RecA^+$ recombinant cosmids, in addition to the vector band, share a fragment of about 8 kb (lanes 1-5). As expected, no DNA homolgy (except for the cosmid DNA) was detected between the fragment inserts of the $RecA^+$ clones and those of an amp^r but MMS-sensitive (MMS^s) clone of the library (lane 6) that was used as negative control. *EcoRI* cleaves pJC859 into two fragments of 5750 and 1850 bp in size (Fig. 1A, lane 7). In the hybridization experiment, however, only the larger fragment shows homology with the *recA* sequence of *A. magnetotacticum* (Fig. 1B, lane 7). This fragment carries about 80% of the *E. coli recA* gene, including and the promoter and the operator sequence (Sancar et al. 1980). When

E. coli sequence was used as probe, only the 8-kb fragment of the RecA$^+$ recombinant cosmids, hybridized with the labeled probe (data not shown).

Restriction mapping of the *A. magnetotacticum recA* gene. The restriction digestion and Southern hybridization analysis described above indicated that all five RecA$^+$ recombinant cosmids shared a fragment of about 8 kb that hybridized with the labeled *E. coli recA* sequence. This fragment was purified and cloned into the *EcoRI* site of pBR322. The recombinant plasmid thus formed was used to transform HB101. All amp^r transformants were found to be MMS^r. When 20 of the amp^r/MMS^r clones were tested for their sensitivity to UV light, all were found to be also UV resistant (UV^r). These results indicated that the 8-kb fragment carries the *recA* sequence of *A. magnetotacticum*.

The 8-kb fragment was digested with *PstI* and/or *ClaI* endonucleases and the resulting fragments were cloned into pBR322 digested with the same endonucleases. Upon screening of the transformants and identification of the fragments that conferred MMS^r and UV^r, the *recA* sequence of *A. magnetotacticum* was localized to a fragment of about 3-kb between *PstI* and *ClaI* restriction sites (Fig. 2). The 3-kb fragment was further purified and ligated with pBR322 digested with *PstI* and *ClaI* endonucleases. The ligated DNA molecules were used to transform HB101. Since *PstI* and *ClaI* digestions inactivate both antibiotic resistance markers of pBR322, transformants were selected for MMS^r and were further tested for UV^r. All MMS^r transformants were found to be UV^r and to carry the plasmid of the expected size. This plasmid construct was designated pNW300.

Complementation studies with *A. magnetotacticum recA* gene. The recombination proficiency of HB101 clones carrying the *recA* gene of *A. magnetotacticum* was determined by measuring the plating efficiency of a *red*$^-$ *gam*$^-$ mutant strain of bacteriophage λ (λ Fec$^-$ phenotype). This mutant phage requires the recombination activity of the RecA protein for its growth in *E. coli* cells (Manly et al. 1969). All clones carrying the *recA* gene of *A. magnetotacticum* supported the growth of λ *red*$^-$ *gam*$^-$ phage. The plating efficiency of the phage on these RecA$^+$ library clones was the same--about 30% of that obtained with strain HB101(pJC859), which carries the *E. coli recA* gene. Strain HB101 alone did not support the growth of λ *red*$^-$ *gam*$^-$, and only few plaques were formed on plates with the lower dilutions of phage.

Because of their deficiency in DNA repair, the *recA* mutant strains of *E. coli* are sensitive to UV light. To see whether the *recA* gene of *A. magnetotacticum* can complement the mutant function, we examined the ability of the RecA$^+$ recombinant cosmids to repair the UV-damaged DNA of their *recA*$^-$ host. In these experiments, *E. coli* strain HB101, which carried pJC859, and a library clone picked at random were used as positive and negative controls, respectively. Quantitative UV survival measurements (Fig. 3) indicated that all recombinant cosmids with the cloned sequence conferred UV^r upon their host. The extent of protection in each case was similar to that confered by pJC859. No protection was detected by a control recombinant cosmid that was amp^r and MMS^s.

The RecA protein of *E. coli* promotes the proteolytic cleavage of the LexA protein which negatively regulates the expression of a number of unlinked chromosomal genes of *E. coli* (Walker 1984). Since LexA is also

the colicin E1 gene repressor, we examined whether the RecA protein of *A. magnetotacticum* promotes the cleavage of the LexA protein and induces the expression of the colicin E1 gene. For this purpose, strains HB101, HB101(pJC859), and HB101(pNW300) were transformed with plasmid pNP12 (Waleh and Johnson 1985). This pBR322—derived plasmid confers resistance to tetracycline and carries the entire colicin E1 operon. Transformants were selected for tetracycline resistance and were tested for colicin production. Of 8 colonies tested, all pNP12-carrying clones of HB101(pNW300) produced colicin. The zones of inhibition produced by these clones, however, were smaller than those produced by HB101 clones carrying plasmids pNP12 and pJC859 (6 mm versus 11 mm). As expected, none of the HB101(pNP12) colonies produced any colicin. One transformant colony from each transformation set was picked and tested for the production of colicin in the presence of mitomycin C. Cultures, grown to midlog phase, were divided in half. To one half, mitomycin C was added at the final concentration of 1 µg/ml; the other half was used as control. After 2 h of incubation, samples were taken, cells were pelleted, and the supernatants were titrated for colicin activity on a colicin-sensitive strain, CL142. The results (presented in Table 1) indicated that the amount of colicin produced by pNP12-carrying strain of HB101(pNW300) was increased 100× upon treatment with mitomycin C. This amount of colicin was, however, tenfold less than the one produced by the pNP12-carrying strain of HB101(pJC859) that carries the native gene. No colicin activity was detected in the HB101(pNP12) culture supernatant.

Protein analysis of strain HB101(pNW300). Soluble protein extracts were prepared from untreated and mitomycin C-treated or UV-irradiated cells of

HB101(pNW300), HB101(pJC859), and HB101; they were electrophoresed in a polyacrylamide gel and stained with Coomassie brilliant blue (Fig. 4). Treatment of HB101(pNW300) with either mitomycin C or UV induced the production of a protein that migrated near the position of *E. coli* RecA protein. This protein was absent in extracts of strain HB101.

Discussion

We have cloned and partially characterized a DNA fragment of the genome of *A. magnetotacticum* that codes for a protein analogous to the *recA* gene product of *E. coli* K12. The screening technique we used was based on heterologous complementation of an *E. coli recA* mutant that was first described by Better and Helinski (1983) for cloning the *recA* gene of *Rhizobium meliloti*. This technique was later used by others (Keener et al. 1984; Ohman et al. 1985; Goldberg and Mekalanos 1986; Koomy and Falkow 1987) to clone analogous *recA* sequences from other bacterial species.

DNA hybridization experiments demonstrate that significant homology exists between the *recA* sequence of *A. magnetotacticum* and that of *E. coli* K12. This homology appears to be mainly at the amino-terminal portion of the two sequences, however, as evidenced by hybridization of probes complementary to the *recA* of *A. magnetotacticum* with the fragment that carried the amino-terminal and nearly 80% of the total *recA* sequence of *E. coli* (Fig. 1).

The RecA protein of *A. magnetotacticum* restored recombination proficiency in the *E. coli recA* mutant host. This was demonstrated by increased plating efficiency of the λ Fec^-, which requires the *recA* function of the host for growth. The lower number of λ plaques observed with the RecA

protein of *A. magnetotacticum* may be due to inefficient expression from the heterologous promoter or, alternatively, due to instability of the RecA protein in a foreign host. Indeed, in crude cell extracts, the RecA protein of *A. magnetotacticum* appears to be unstable and degrades rapidly upon short-term storage.

The RecA protein of *A. magnetotacticum* increased cell viability of the host to wild-type levels in response to UV exposure. HB101 cells with *recA* gene of *A. magnetotacticum* were as UV resistant as those carrying the native sequence. Similar levels of protection have been reported in heterologous complementation studies with the RecA proteins of *Proteus vulgaris, Shigella flexneri, Erwinia carotovara*, and *E. coli* B/r (West et al. 1983; Keener et al. 1984). However, RecA protein of *R. meliloti* has only partially suppressed the UV sensitivity of an *E. coli recA* mutant (Better and Helinski 1983).

The most interesting results were the findings that the *recA* of *A. magnetotacticum* not only recognizes the LexA protein of *E. coli* but that the *recA* itself may be regulated by this repressor molecule. Our colicin induction experiments clearly demonstrate that the RecA of *A. magnetotacticum* promotes the cleavage of the LexA repressor, which leads to the derepression of the colicin E1 operon. Moreover, the finding that both UV and mitomycin C increased the level of the RecA protein of *A. magnetotacticum*--a response observed with *E. coli* RecA--strongly suggest that the cloned *recA* gene is regulated by the LexA protein of *E. coli*. Whether a LexA-like protein exists in the native host and whether it regulates the expression of the *recA* gene of *A. magnetotacticum* remains to be demonstrated.

Our results and those of the other investigators discussed above provide compelling evidence that the RecA protein is structurally and functionally preserved among gram negative bacteria. DNA and amino acid sequence analysis

of RecA from various species should provide valuable information about the history of the evolution of this important multifunctional bacterial protein. DNA sequence analysis of the *recA* gene of *A. magnetotacticum* is currently in progress.

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Fig. 1A, B. *EcoRI* digestion patterns of RecA$^+$ recombinant cosmids (lanes 1-5), a control RecA recombinant cosmid (lane 6), and pJC859 (lane 7). The marker fragments, 1 kb ladder obtained from BRL, are shown in lane M. B. Southern blot hybridization of DNA fragments shown in A using the *EcoRI* digested and ^{32}P-labeled DNA fragments of one of the RecA$^+$ recombinant cosmids (shown in lane 1) as probe. The vector band is indicated by ⟶ and the 8.0 kb *EcoRI* fragment shared by all RecA$^+$ recombinant cosmids is shown by ◇ .

Fig. 2. Preliminary restriction map of the 8.0 kb *EcoRI* fragment carrying the *recA* gene of *A. magnetotacticum*. The dashed line shows the approximate location of the *recA* gene as suggested by subcloning experiments described in the text.

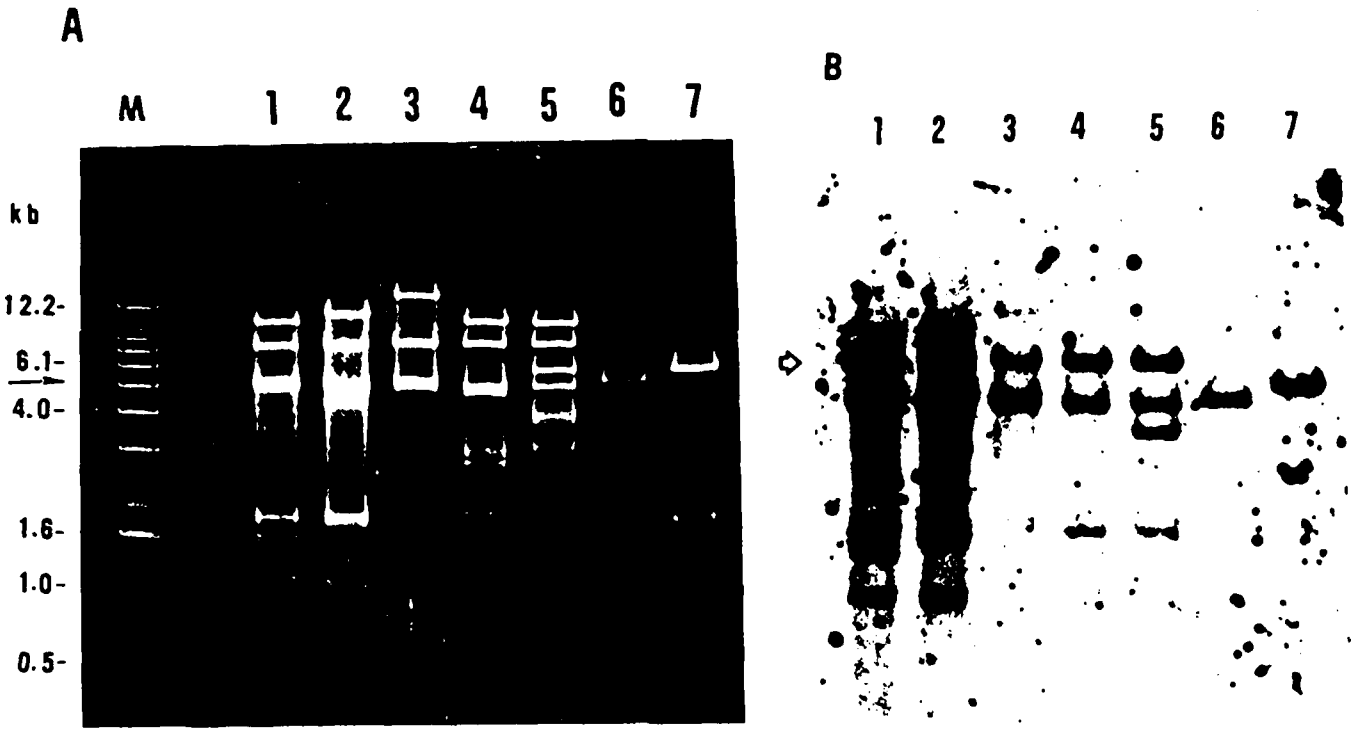
Fig. 3. UV survival of cells carrying the *recA* gene of *A. magnetotacticum*. Symbols: x, ▲, ■, ▼, and ●, RecA$^+$ library clones; ○, HB101(pJC859); and □, a RecA$^-$ library clone.

Fig. 4. Protein analysis of plasmid pNW300. Cells were grown in minimal medium to midlog phase when they were treated with mitomycin C at 1 µg/ml, or UV irradiated for 30 seconds. After two hours of incubation at 37°C, cells were pelleted and treated as described under Materials and Methods. Lanes 1-3, 4-6, and 7-9 show protein samples from control, mitomycin C-treated, and UV-irradiated cells of HB101, HB101(pNW300), and HB101(pJC859), respectively. Molecular weight protein standards are given × 10^{-3}.

Table 1. Colicin production after induction of pNW300 and pJC859 with mitomycin C (MC).

Strains carrying plasmid pNP12	Colicin titer	
	-MC	+MC
HB101 (pNW300)	0.01	1
HB101(pJC859)	0.01	10
HB101	0	0

Results represent colicin titer $\times 10^{-2}$. The colicin titer is defined as the reciprocal of the last dilution giving noticeable clearing of the indicator lawn.


A
M
1
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12.2-
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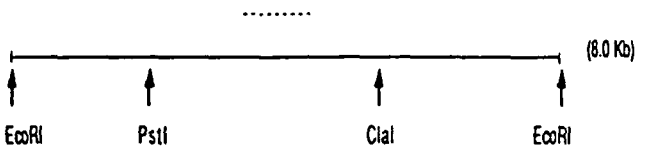
(8.0 Kb)
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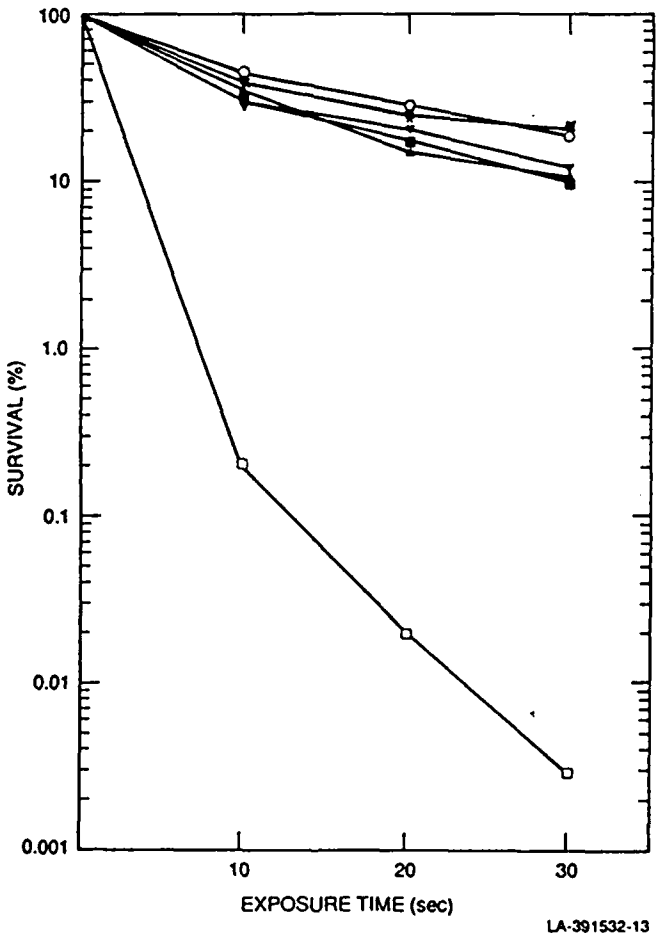
100
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0.01
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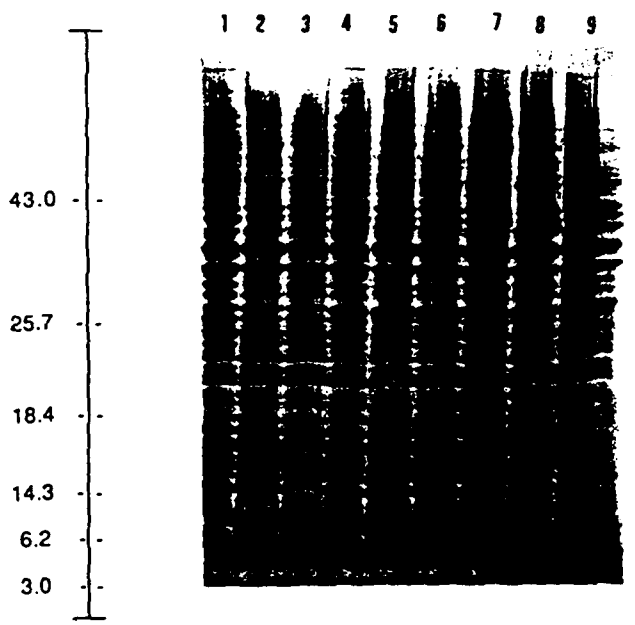
1 2 3 4 5 6 7 8 9
43.0
25.7
18.4
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