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Low-molecular-mass RNA fingerprinting of bacteria by capillary electrophoresis using entangled polymer solutions

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Abstract

Low-molecular-mass RNA (LMM RNA) fingerprinting uses the stable LMM RNA fraction (transfer RNA and 5S ribosomal RNA), with a size range of 70–135 nucleotides, from single bacterial strains for a genotypic identification and classification of bacteria. A novel application of capillary electrophoresis (CE) using entangled polymer solutions to separate LMM RNA to generate LMM RNA fingerprints was developed. Buffers containing 0.5% hydroxypropylmethylcellulose (HPMC) as sieving additive were employed to obtain improved peak resolution and good reproducibility. CE using 0.5% HPMC was suitable for routine analysis of LMM RNA, providing high resolution, reproducibility, system automation and high stability of the buffer. The relative standard deviation of the migration time for twenty successive runs was 0.5%. In addition, the variation of the resolution reproducibility of two separate buffer preparations was not greater than 5%. The limit of detection of RNA is ca. 0.02 μg per peak and separations can be performed using RNA samples as dilute as 10 μg/ml. One disadvantage of this system in the short lifetime of the capillary coating; DB-1 capillaries can be used for only 30–50 runs. LMM RNA fingerprints obtained with this system were compared with samples separated by polyacrylamide slab gel electrophoresis. The use of CE has the potential to provide a rapid and convenient way of identifying bacterial strains.

1. Introduction

The automation of conventional electrophoresis techniques is currently of great importance and interest owing to their universal use in a range of scientific applications. The classical manual separation methods applied in medical, molecular biological and genetic studies almost exclusively use slab polyacrylamide and agarose gel electrophoresis. Capillary electrophoresis (CE) employs the separation mechanisms of conventional electrophoresis in a capillary format and offers the possibility of quantitative analysis and automation. Recently, instead of classical electrophoresis, CE has been applied in many applications owing to its many advantages such as automated sample handling, reduced analysis time, improved on-line detectability without the need for staining procedures, small buffer requirements and samples and ease of use [1].

Initially, the use of capillary gel electrophoresis (CGE) was expected to offer a realistic alternative to the conventional slab gel electrophoresis [2–4]. Although excellent resolution has been demonstrated using CGE in the separation

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of DNA molecules and synthetic oligonucleotides [2–4], the manipulations required to cast gels in capillaries, the short capillary lifetimes and their low reproducibility would appear to limit the use of CGE for routine analysis.

In this respect, a possible alternative to CGE employs the use of CE using entangled polymer solutions [5–11]. The inclusion of linear polymers in the separation electrolyte generates a molecular sieving effect. The resolution of DNA fragments in the example of ΦX-174 RF DNA/*Hae*III by CE using entangled polymer solutions is comparable to that of CGE [10]. Additionally, CE using entangled polymer solutions represents a simple and inexpensive separation technique, suitable for routine analysis, owing to replaceable polymer separation matrices, and provides reproducible separations on columns that are easily refilled.

Many workers have reported the increasing applicability of CE using entangled polymer solutions for the analysis of synthetic oligonucleotides, PCR products and DNA restriction fragments [5–11]. Analysis of polymerase chain reaction heteroduplex polymorphism [9] and restriction fragment length polymorphism [10] are carried out using this method as diagnostic tools for clinical assays. Good results have also been reported in the automated ribosomal DNA fingerprinting of PCR products for taxonomic studies of fungi [11].

We have previously reported [12] the separation of low-molecular-mass (LMM) RNA by CE using entangled polymer solutions. LMM RNA profiling provides a genotypic fingerprint technique for identifying bacteria [13]. This technique is of particular importance for molecular ecology and taxonomic studies, and can also be applied directly to analyses of environmental samples [14]. This RNA fingerprinting method uses the bacterial LMM RNA (small tRNAs, large tRNAs and 5S rRNA in a size range between 70 and 135 nucleotides), enabling strains to be classified to the genus and species level on the basis of species- and genus-specific tRNA bands [13,15].

The standard established technique for LMM RNA profiling employs slab polyacrylamide gel electrophoresis (PAGE) using 55 cm length gels with high resolution [15]. However, a more rapid, automated technique is needed for LMM RNA profiling in ecological studies. The use of CE with 0.5% HPMC as sieving additive in buffers resolved 5S rRNA and tRNAs, even when they possessed only different secondary structure or small differences in length (1–5 nucleotides) [12]. In order to determine the reliability of this methodology, we investigated the optimum conditions in terms of sample size, detectability, reproducibility and stability of the sieving buffer, as well as of the DB-1 capillaries. We report on the first analysis of LMM RNA fingerprints using a CE-based identification of bacterial strains from different genera and we compare them with LMM RNA fingerprints obtained by conventional slab PAGE.

2. Experimental

2.1. Organisms and growth conditions

The following strains were obtained through culture collections (listed as strain source and catalogue number): *Alcaligenes eutrophus* DSM 531^T, *Hypomicrobium facilis* ATCC 27485^T, *Escherichia coli* DSM 30083^T, *Pseudomonas alcaligenes* LMG 1224^T and *Pseudomonas fluorescens* DSM 50090^T (T = type strain of the species). Strains were grown on relevant standard culture media, harvested by centrifugation at 18 000 *g* for 5 min and stored at −20°C prior to RNA extraction.

2.2. Materials

All chemicals were of analytical-reagent grade or of high purity. All water used was ultra-pure, doubly distilled and deionized. Tris(hydroxymethyl)aminomethane (Tris), ammonium peroxodisulphate and N,N,N′,N′-tetramethylethylenediamine (TEMED) were obtained from Sigma (St. Louis, MO, USA). Acrylamide was purchased from Amresco (Solon, OH, USA) and 3-(trimethyoxysilyl)propylmethacrylate (Bind Silane) from LKB (Bromma, Sweden).

Ethylenediaminetetraacetic acid (EDTA) and boric acid were obtained from Riedel-de Haën (Deelze, Germany). The polymer hydroxypropylmethylcellulose (HPMC), with a viscosity rating of 4000 cP for a 2% solution at 25°C, was obtained from Sigma.

A tRNA mixture from *Escherichia coli* MRE 600, containing 5S rRNA and tRNAs, was purchased from Boehringer (Mannheim, Germany). This tRNA mixture was used as a standard LMM RNA mixture.

Open-tubular capillaries coated with DB-1 were purchased from J&W Scientific (Folsom, CA, USA).

2.3. Equipment

A SpectraPhoresis 500 CE system (Spectra-Physics, Fremont, CA, USA) was used in the negative polarity mode (anode at the detection side) for all separations. Temperature control was achieved with a Peltier cooling system on the SpectraPhoresis 500. Electrokinetic injections were carried out for RNA-containing samples. The internal standard 2′-deoxyadenosine 5′-triphosphate (dATP) was injected hydrodynamically for 0.1 min in a double injection mode before sample injection. Nucleic acids were detected at 260 nm using a UV detector.

2.4. RNA extraction

Total RNA was extracted as described previously [15] from 10 mg of bacterial biomass (dry mass) with hot sodium dodecyl sulphate–phenol, purified in several steps and finally precipitated with ethanol.

2.5. Sample treatment

Crude extracts of total bacterial RNA were treated either by ultrafiltration or by Qiagen column chromatography for removal of large RNA fragments (16S rRNA and 23S rRNA) and other high-molecular-mass components (such as polysaccharides).

Ultrafiltration

Ultrafiltered samples were obtained by centrifugation at 6000 *g* for 35 min at 4°C in Centricon-100 concentrators (Amicon, Beverly, MA, USA) to remove the high-molecular-mass RNAs.

Qiagen column chromatography

Crude extracts containing 60 μg of total RNA were subjected to Qiagen column chromatography (Qiagen tip 20; Qiagen, Chatsworth, USA), as recommended by the manufacturer.

2.6. Capillary electrophoresis using entangled polymer solutions

DB-1 open-tubular capillaries, 70 cm long (62 cm effective length) × 100 μm I.D. with a coating of 0.1 μm dimethylpolysiloxane, were used without modification. DB-1 capillaries (70 cm) were filled automatically with the appropriate polymer sieving buffer for 20 min. Between runs, the capillaries were washed for 3 min at 60°C in the following sequence: water, methanol, 2-propanol, methanol, water. For monitoring the coating quality we have used the LMM RNA mixture from *E. coli* MRE 600. Depending on the conditions employed, capillaries were used for 30–50 runs.

The polymer sieving buffer contained 350 m*M* Tris–borate, 2 m*M* EDTA, 7 *M* urea (pH 8.6) and 0.5% (w/w) HPMC, prepared as reported previously [12].

RNA samples were prepared at a concentration of 100 μg/ml in 40 m*M* Tris–HCl buffer–1 m*M* EDTA (pH 5.0). Typically, the entire available volume of samples was 20 μl and many runs could be carried out. The crude extract (500 μg/ml) was five times more concentrated than the partially purified samples and was injected at −5 kV for 20 s. Partially purified samples were injected at −15 kV for 20 s at a concentration of 100 μg/ml. dATP was used as an internal standard for analysis of LMM RNA profiles. Before sample injection, the injection side of the capillary was washed twice with water for 0.1 min. All separations were performed at 20°C.

To determinate the electroendosmotic flow

(EOF), a neutral marker (0.5% mesityl oxide) was injected for 0.1 min hydrodynamically. The separation was carried out in the positive polarity model.

2.7. Conventional gel electrophoresis

Separation of LMM RNA was carried out under high power by denaturing PAGE [16]. Dried samples of LMM RNA (3 μg) were suspended in 4 μl of a loading solution (300 mg/ml sucrose, 460 mg/ml urea, 10 μl/ml 20% SDS, 1 mg/ml bromophenol blue, 1 mg/ml xylene cyanol, all substances dissolved in TBE buffer) and loaded on polyacrylamide gels {gel size 550 × 170 × 0.4 mm, 10% acrylamide [acrylamide–N,N-methylenebisacrylamide (28.8:1, w/w), 7 *M* urea, in TBE buffer [100 m*M* Tris–83 m*M* boric acid–1 m*M* EDTA (pH 8.5)]} that were bound to a carrier glass plate and run in a high-power electrophoresis unit (2010 Macrophor electrophoresis unit and 2297 Macrodrive 5 power supply; LKB–Pharmacia, Bromma, Sweden). Gels were run at 60°C and at constant power that was increased stepwise from 40 to 160 W during the 3-h run. RNA bands were revealed by a modified ammoniac silver staining procedure [15]. Gel scans were performed using a transmission densitometer (Elscript 400; Analysentechnik Hirschmann, Taufkirchen, Germany) at a wavelength of 546 nm. A detailed description of the conventional electrophoresis of LMM RNA has been given previously [15].

3. Results and discussion

3.1. Optimization of sample injection and detectability

To determine the optimum amount of LMM RNA that gives the best detectability using electrokinetic injection, a LMM RNA mixture from *E. coli* MRE 600 (1–100 μg/ml) was injected at either −10 kV for 15 s or −20 kV for 30 s and analysed using 0.5% HPMC sieving buffer in DB-1 capillaries. Compared with the classical gel electrophoresis method, electrokinetic injection uses relatively small amounts of sample. Sample loading is dependent on the electroosmotic flow, sample concentration and sample mobility. Variations in conductivity, which can be due to matrix effects, result in differences in voltage drop and amount loaded. In this experiment, we examined the response between sample concentration and peak area without considering the other factors mentioned above. Determination of the absolute amount of sample used for each run was not carried out. A linear relationship was obtained between peak area and sample concentration in the range 10–100 μg/ml RNA (Fig. 1A).

Using electrokinetic injection at −10 kV for 15 s, no distinct profile was produced at concentrations up to 10 μg/ml (Fig. 1B). Therefore, the injection parameters were altered to −20 kV for 30 s for low concentrations of LMM RNA in the range 5–10 μg/ml (Fig. 2). Additionally, the profile resolution using sample concentrations of 5 μg/ml was not satisfactory because of the high signal-to-noise ratio. Increasing the voltage and injection time (−30 kV for 30 s) did not improve the detectability of the RNA at concentrations lower than 5 μg/ml.

The limit of detection with injection at −20 kV for 30 s at the lowest detectable concentration (10 μg/ml) is ca. 0.02 μg of total RNA per peak (Fig. 2). However, concentrations between 20 and 50 μg/ml are recommended for good resolution with injection at −10 kV for 15 s. In comparison with CE, 2 μg of total RNA per sample are normally used in conventional slab gels per run. Hence CE is more sensitive than slab gel electrophoresis. A further increase in sensitivity could be obtained using fluorescence detection (limit of detection ca. 1 pg DNA per band), as often reported [8,17–19]. This could be of particular importance when analysing environmental samples that contain low concentrations of RNA.

3.2. Coating stability

The stability of the coating of DB-1 capillaries in CE using a sieving buffer containing 0.5%

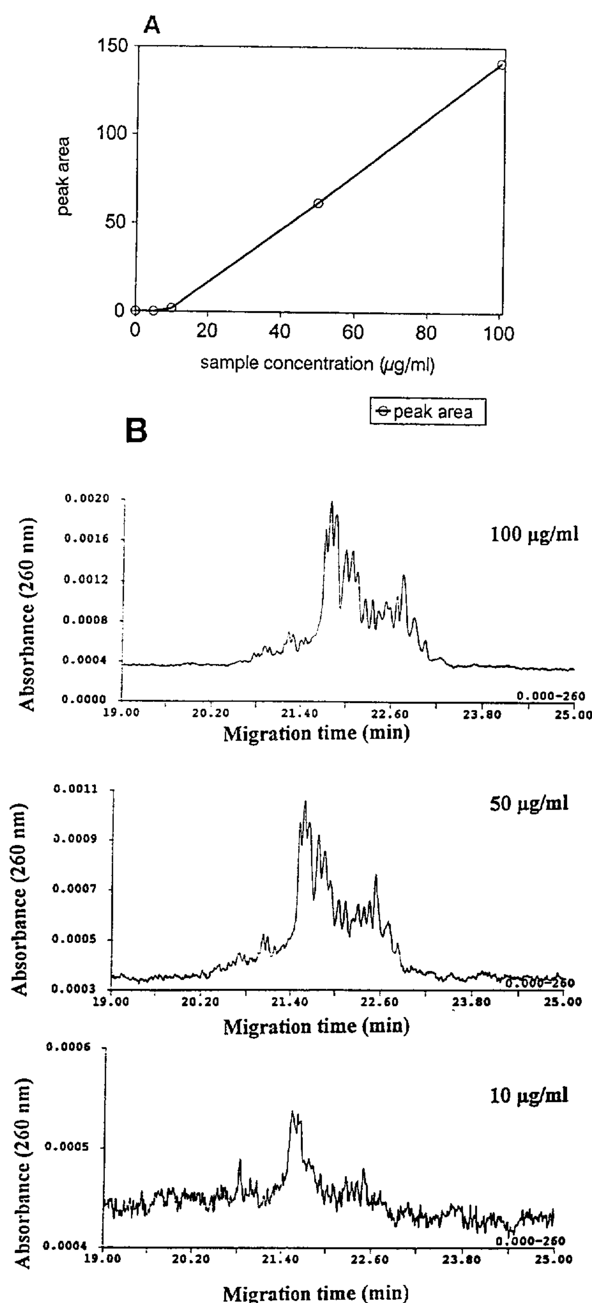


Fig. 1. (A) Sample concentration of LMM RNA plotted versus peak area. (B) Effect of total LMM RNA concentration on detectability of LMM RNA profiles analysed by CE using entangled polymer solutions. Sample, LMM RNA mixture from *E. coli* MRE 600; buffer, 350 m*M* Tris–borate–2 m*M* EDTA–7 *M* urea–0.5% HPMC (pH 8.6); capillary, DB-1, 70 cm (62 cm effective length) × 100 μm I.D., 0.1 μm thickness; injection, −10 kV, 15 s; field strength, 330 V/cm; detection, 260 nm.

HPMC was determined over the course of 150 runs using a standard LMM RNA mixture from *E. coli* MRE 600. Despite extensive washing (see Experimental) between runs, a gradual loss of resolution was observed in the LMM RNA profile. The first loss of resolution was observed (Fig. 3) after 50–70 successive runs and total loss after 150 successive runs and, despite extensive washing, regeneration of the column was not possible. In contrast to washing the capillary between runs, when the capillary was not washed the resolution decreased gradually up to 30–40 runs and then rapidly decreased, with a total irreversible loss of resolution occurring between 40 and 50 runs. Irreversible loss of resolution with DB-1 capillaries after 30–50 successive injections has also been reported in the separation of unpurified PCR products [11].

In contrast to samples containing complex RNA mixtures, samples with only one or two components (e.g., LMM RNAs or oligonucleotides) are well resolved, and for these small biomolecules (20–120 nucleotides long) a capillary could be used for over 150 injections (data not shown). However, the migration times of the components decreased and the relative standard deviations (R.S.D.s) increased with successive runs, indicating changes to the coating.

Similar results (not presented) were also obtained using other DB capillaries (DB-17, DB-Wax) that are coated with different phases relative to their matrix, polarity and hydrophobicity. These other DB capillaries produced LMM RNA profiles similar to that of DB-1, and a first loss of resolution was obtained after 50–70 successive runs in all cases.

Because none of the capillaries gave a detectable EOF (a measure of coating instability), even after 150 successive runs, the observed loss of resolution in the separation of LMM RNA profiles is presumably not dependent on coating deterioration or loosening from the capillary wall. Additionally, when negatively charged samples (such as RNA) are loaded, no detection should be possible when the column coating is detached or degraded owing to the negative polarity, suggesting that resolution is detrimentally affected, probably by the successive ac-

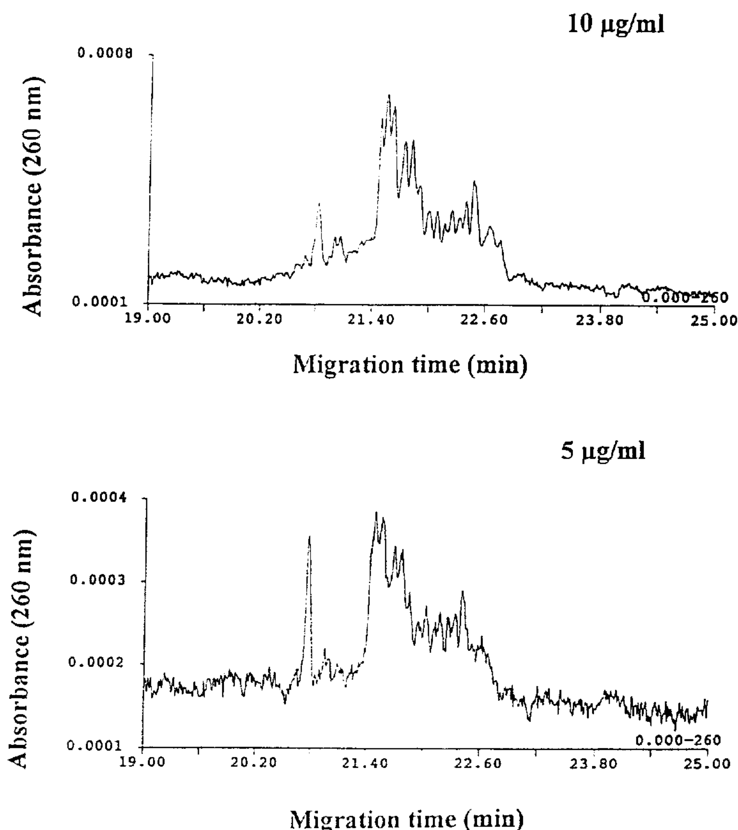


Fig. 2. Effect of total LMM RNA concentration on detectability of LMM RNA profiles analysed by CE using entangled polymer solutions fro the separation of the LMM RNA mixture from *E. coli* MRE 600. Injection, −20 kV, 30 s; other conditions as in Fig. 1.

cumulation of HPMC on the coated capillary wall.

The successive accumulation of HPMC was previously described by Bello et al. [20] as a "dynamic coating", a successive non-covalent coating of methylcellulose on the capillary wall of uncoated capillary columns. The polymer molecules in the layer of the "dynamic coating" near the capillary wall undergo an orientation or conformation change, which results in a reduced fluid viscosity in this region, relative to the bulk [20]. In the case of the DB-1-, DB-17- and DB-Wax-coated capillaries, a similar behaviour of "dynamic coating" may also be the cause of the progressive loss of resolution with each successive run, the possible decrease in polymer viscosity resulting in a decrease in the resistance of the separated RNA molecules through the polymer network.

3.3. Reproducibility

In order to define the reproducibility of CE using entangled polymer solutions containing 0.5% HPMC in the separation of LMM RNA profiles, 55 successive runs were performed with a new DB-1 capillary. The reproducibility of relative migration time was determined by the migration times of the first and last peak of the standard LMM RNA mixture of *E. coli* MRE 600 (Fig. 4). The run-to-run reproducibility decreases with increasing run number. The R.S.D.s of the migration time of the first and last fragments were for 20 successive runs 0.51 and

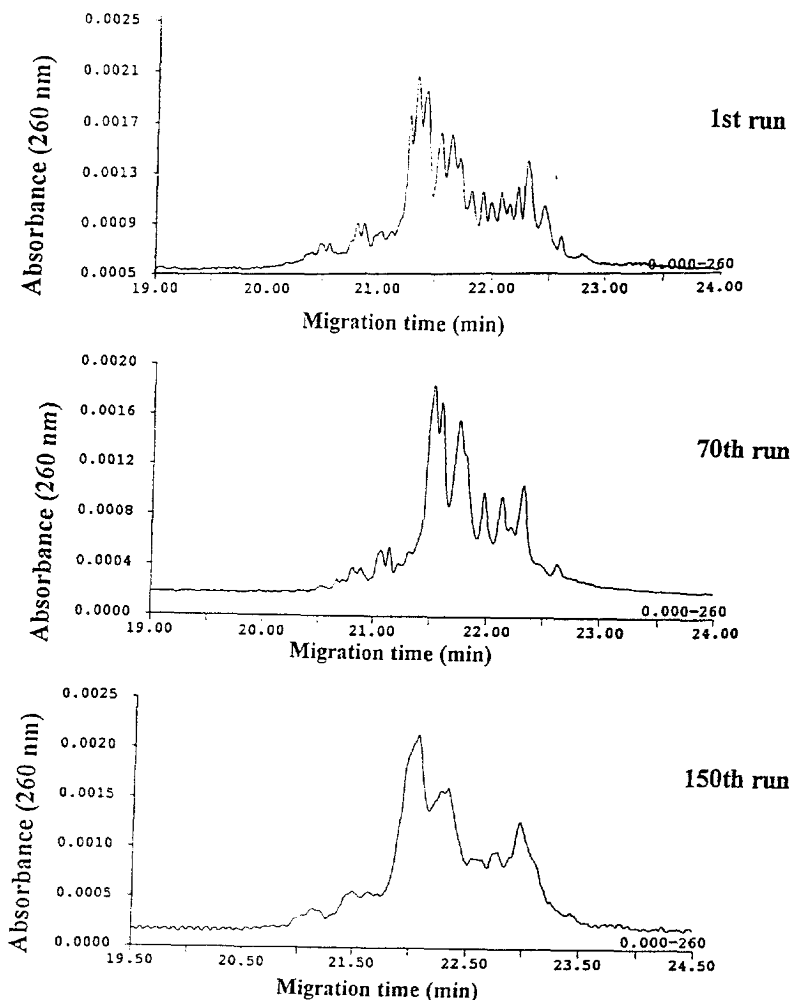


Fig. 3. Loss of resolution through capillary instability determined after 150 successive runs by CE using entangled polymer solutions. Sample, LMM RNA mixture from *E. coli* MRE 600 (100 μg/ml); conditions as in Fig. 1.

0.5%, for 30 runs 0.86 and 0.76% and for 55 runs 1.3 and 1.25% (Table 1). The loss of migration time reproducibility is consistent with the previously mentioned deterioration in coating stability with successive runs (see above).

3.4. Buffer stability and reproducibility in terms of profile resolution

Reproducibility in terms of profile resolution is paramount in separations of LMM RNA fingerprints because the primary goal is the identification of bacteria using a database. In order to determine the reproducibility of buffer preparation in terms of profile resolution and migration time variation, two separate preparations of the 0.5% HPMC sieving buffer [HPMC (1) and HPMC (2); Fig. 5] were tested. The buffer reproducibility was determined for the separation of the LMM profile of *Pseudomonas alcaligenes* LMG 1224^{T} over a period of 1 month during 30 runs. Runs were carried out daily using the same DB-1 capillary. In order to ensure coating stability, a new DB-1 capillary was used.

The stability of the resolution of the profiles was evaluated with software designed for capil-

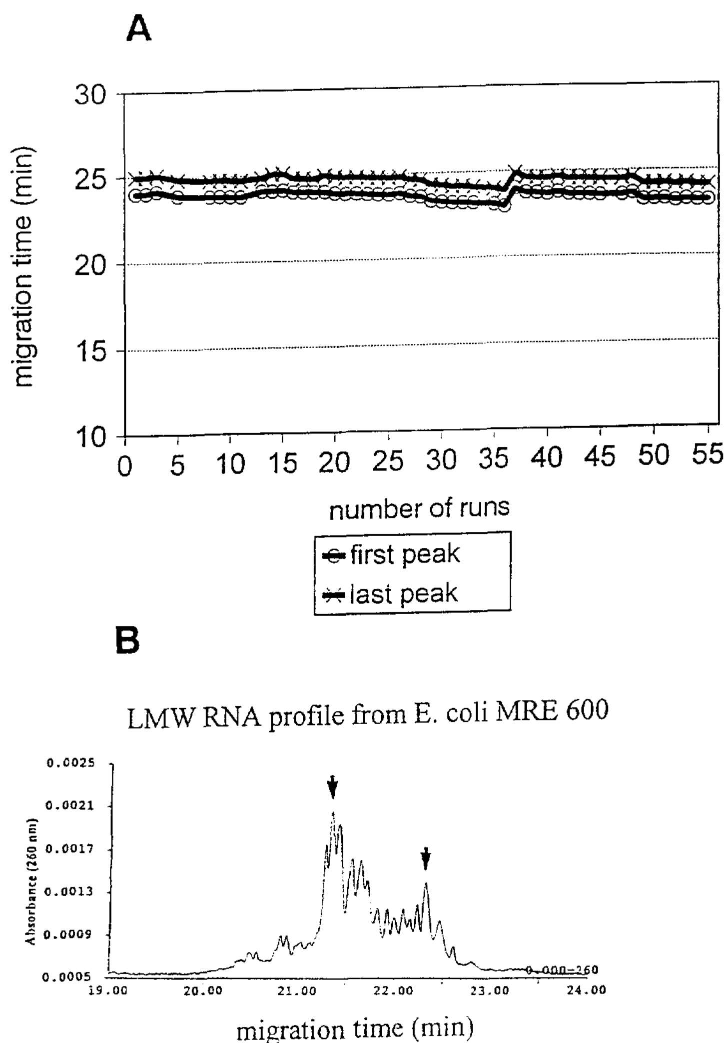


Fig. 4. (A) Migration time reproducibility of two fragments [arrowed, see (B)] of the standard LMM RNA mixture from *E. coli* MRE 600 by CE using entangled polymer solutions. The mobility has not been corrected with an internal standard. Sample, 100 μg/ml; experimental conditions as in Fig. 1.

lary files (GelManager for CE files V.1.5; Bio-Systematica, Devon, UK). The CE files were imported into the GelManager software after conversion into ASCII files. Similarity of the profiles was calculated by the correlation of whole profiles by the Unweighted Pair Group Method Using Averages (UPGMA).

The 0.5% HPMC sieving buffer, prepared at different times over a 1-month period gives a high reproducibility with correlation coefficients in the range 0.94–1.0 between different runs with the same sample using the same capillary over a period of 1 month (Fig. 5). The correlation between two different buffer preparations is 0.94 by the second day after preparation and 1.00 by the 30th day after preparation (Fig. 5). Increasing storage of the 0.5% HPMC sieving buffer seems to reduce small differences in the

Table 1
Reproducibility of migration time of two peaks in the LMM RNA mixture from *E. coli* MRE 600 by capillary electrophoresis using entangled polymer solutions containing 0.5% HPMC as sieving additive

No. of runs	Migration time (min)	
	First peak	Last peak
1	23.98	24.95
5	23.85	24.81
10	23.78	24.74
15	24.12	25.09
20	23.89	24.86
Average	23.95	24.87
S.D.	0.123	0.125
R.S.D. (%)	0.51	0.50
25	23.75	24.73
30	23.29	24.26
Average	23.84	24.78
S.D.	0.205	0.189
R.S.D. (%)	0.86	0.76
35	23.08	23.99
40	23.65	24.56
45	23.56	24.50
50	23.28	24.21
55	23.21	24.14
Average	23.65	24.58
S.D.	0.312	0.308
R.S.D. (%)	1.3	1.25

Sample, 100 μg/ml; buffer, 350 m*M* Tris–borate–2 m*M* EDTA–7 *M* urea–0.5% HPMC (pH 8.6); capillary, DB-1, 70 cm (62 cm effective length) × 100 μm I.D., 0.1 μm thickness; injection, −10 kV, 15 s; field strength, 330 V/cm; detection, 260 nm.

resolution of separate buffer preparations. According to the instructions of the GelManager software program, the correlation values should be >0.95 between repeated runs with the same sample.

In addition, the R.S.D. of the migration times of the components of the same sample was in the range of 1.5–1.85% between two separately prepared buffers. The 0.5% HPMC sieving buffer seems to be reproducible in terms of profile resolution but with small variations in terms of migration times.

3.5. Sample preparation

The influence of sample preparation and purity of the extracts on the LMM RNA profile from *E. coli* DSM 30083^{T} was investigated. Fig. 6 shows the profiles obtained from (A) crude extract, (B) an ultrafiltered sample and (C) a sample treated by Qiagen column chromatography. Profiles obtained from crude extracts or ultrafiltrated samples (Fig. 6A and B) do not show any major differences. However, the Qiagen-treated sample (Fig. 6C) shows some differences in resolution in terms of peak height and partial overlapping of peaks (Fig. 6C). The differences obtained may be due to the absence of some components in the Qiagen-treated sample that would otherwise influence the changes in conformation of the LMM RNA in the electric field and their interaction with the polymer network. In contrast, the same samples (crude extract, ultrafiltered and Qiagen-treated samples) when analysed by conventional gel electrophoresis did not show any differences in resolution (data not shown).

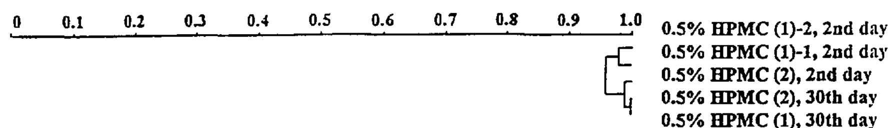
In summary, profiles produced from crude extracts and ultrafiltered samples could be obtained with high resolution using CE. However, the use of crude extracts could have an adverse effect on the coating lifetime because of the large RNA fragment (16S and 23S rRNA) present, or other impurities (e.g., polysaccharides).

3.6. Comparison of CE and slab PAGE using LMM RNA from bacteria of different genera

LMM RNA profiles of four type strains from different genera of bacteria belonging to three different subgroups of the Proteobacteria, *Alcaligenes* (beta), *Hyphomicrobium* (alpha), *Escherichia* (gamma) and *Pseudomonas* (gamma subgroup) were generated by CE using entangled polymer solutions (Fig. 7) and by slab PAGE (Fig. 8).

Consistent with a previous report [12], profiles generally produced by CE using entangled polymer solutions differed from those obtained by slab gel electrophoresis (compare Figs. 7 and 8). The differences between CE and conventional

Dendrogram of clustered pattern



Correlation of profiles

0.5% HPMC (1)-2, 2nd day	100				
0.5% HPMC (2), 2nd day	96	100			
0.5% HPMC (1)-1, 2nd day	98	94	100		
0.5% HPMC (2), 30th day	96	99	95	100	
0.5% HPMC (1), 30th day	97	98	97	100	100

Sample: LMW RNA profile from *Ps. alcaligenes* tl LMG 1224^T

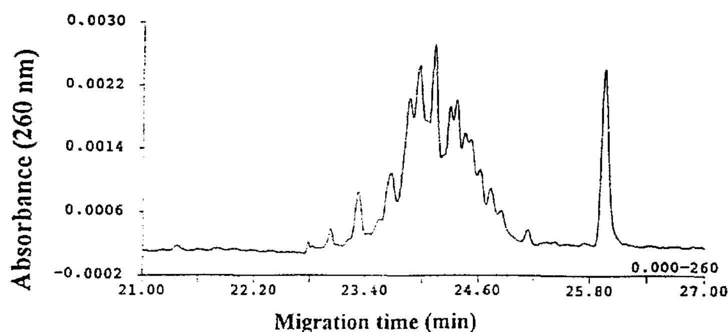


Fig. 5. Stability and reproducibility of the 0.5% HPMC polymer buffer over a period of 1 month in terms of profile resolution by the separation of LMM RNA profile from *Pseudomonas alcaligenes* LMG 1224^T. Sample, 100 μg/ml; injection, −20 kV, 30 s; other conditions as in Fig. 1. Calculation of clusters and correlation of profiles using GelManager software. 0.5% HPMC (1) and (2), two separate buffer preparations; 0.5% HPMC (1)-1 and (1)-2, repeated runs with the same buffer preparation.

electrophoresis are expected, since conventional gel electrophoresis functions under denaturing conditions and separation is based on differences in size and charge, whereas in CE using entangled polymer solutions, electrophoresis is carried out under non-denaturing conditions and molecules are separated on the basis of size, spatial structure, changes in conformation and interactions with the polymer network [12,21]. One notable example is that, in contrast to PAGE, 5S rRNA molecules analysed by CE with the same length (120 nucleotides) from *E. coli* and *Ps. fluorescens* show different separation behaviours, probably because of their spatial structures and the interactions with the polymer network under the non-denaturing separation conditions.

In the RNA profiles produced by CE, 5S rRNA migrates very differently to the other tRNA molecules. Typically, for 5S rRNA, a distinguishable separation from other tRNA molecules is observed as a single peak (Fig. 7). This is not surprising, since in most bacteria only one kind of molecule is expected, and 5S rRNA molecules have a different size to the bulk of residual tRNA molecules in the cell of at least more than 10 nt [15].

In contrast to 5S rRNA, in any given strain,

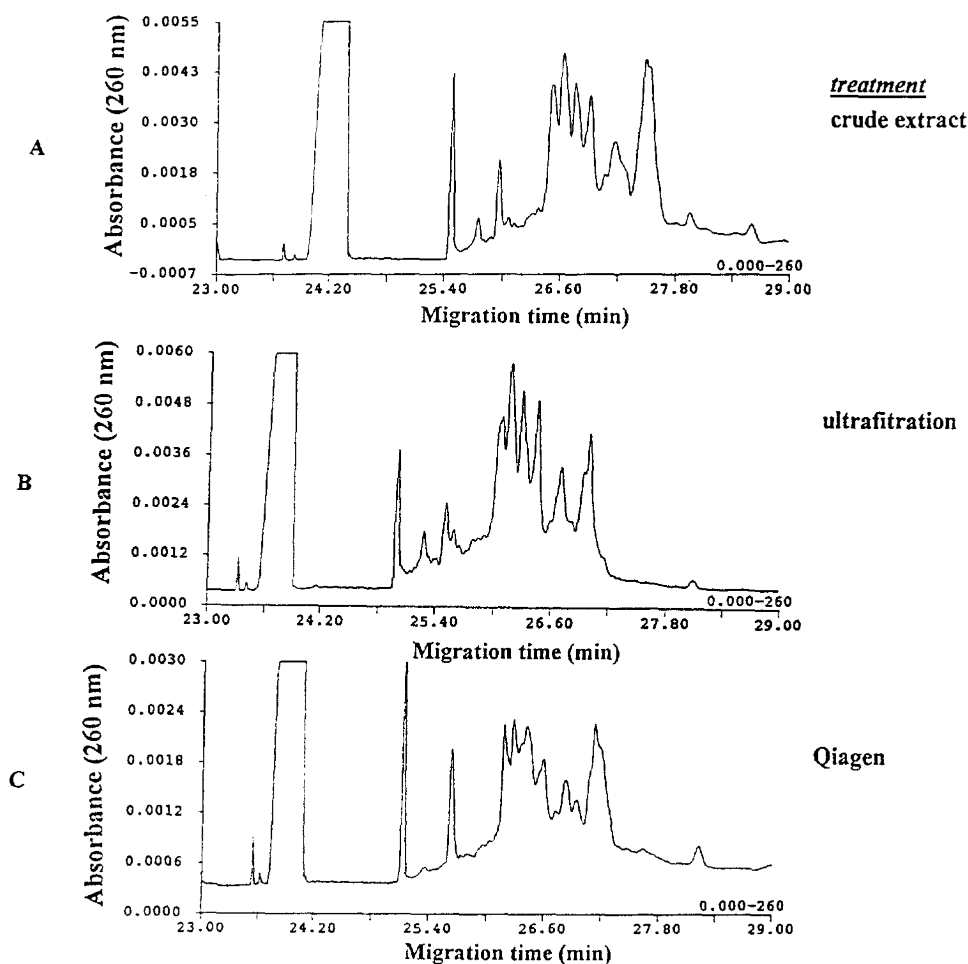


Fig. 6. Influence of sample treatment on LMM RNA profiles produced by CE using entangled polymer solutions. Sample, LMM RNA profile from *E. coli* DSM 30083^T; (A) crude extract (500 μg/ml); (B) ultrafiltered sample (100 μg/ml); (C) Qiagen-treated sample (100 μg/ml). Injection, (A) −5 kV, 20 s; (B) and (C) −15 kV, 20 s. Other conditions as in Fig. 1.

tRNA constitutes a heterogeneous population (60 different tRNA molecules are possible) and, not surprisingly, CE produces a complex multi-peak profile. However, there is not complete resolution of the different tRNA moities, probably owing to the tRNAs often having the same or similar nucleotide length.

Despite the differences in the way RNA is separated by CE and PAGE, there are some similarities in the profiles produced by these techniques, particularly for the 5S rRNA region. For example, the CE-separated profile of *H. facilis* exhibits a double band in the 5S rRNA region (Fig. 7) and this is also observed in the slab gel electrophoresis profile from *H. facilis* (Fig. 8).

Because the tRNA composition is individual for every bacterial strain [15], an analysis of a strain's tRNA composition can provide a fingerprint to aid in the identification of that strain. The tRNA analysis of four strains presented in Figs. 7 and 8 produced different tRNA profiles for each strain (fingerprints) by both conventional slab PAGE and CE. It is notable, however, that the CE-generated tRNA profiles (fingerprints) of the four strains are more dissimilar

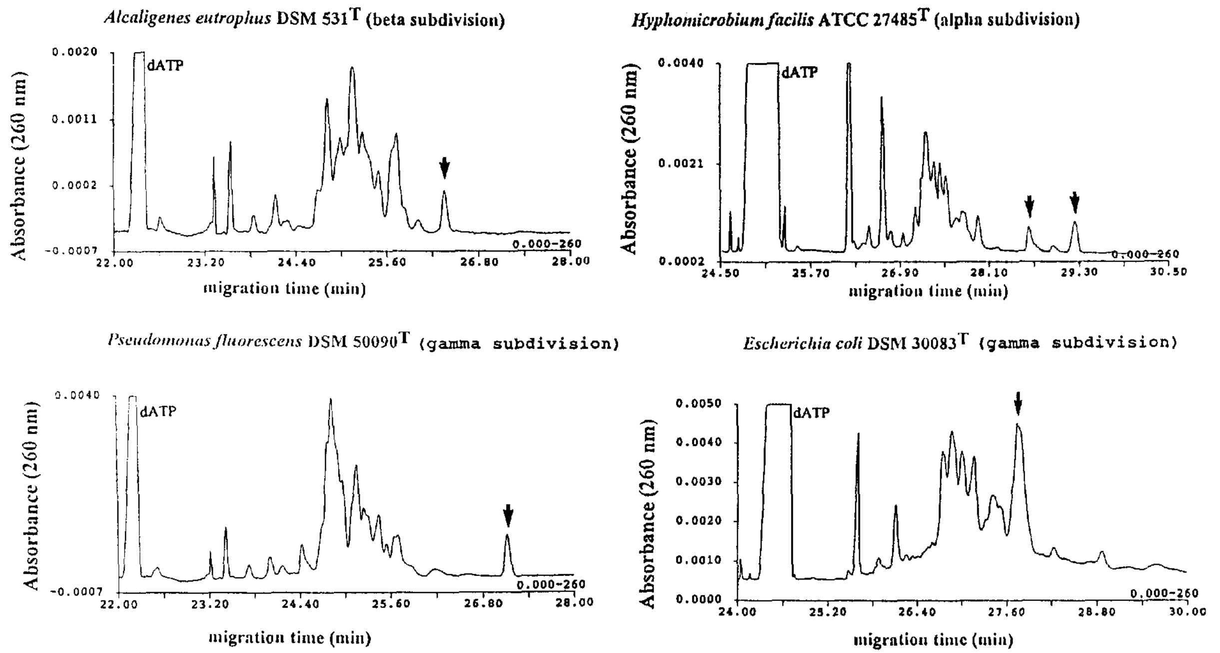


Fig. 7. CE-based LMM RNA fingerprints of bacteria from different genera. 5S rRNA peaks are arrowed. Experimental conditions as in Fig. 1.

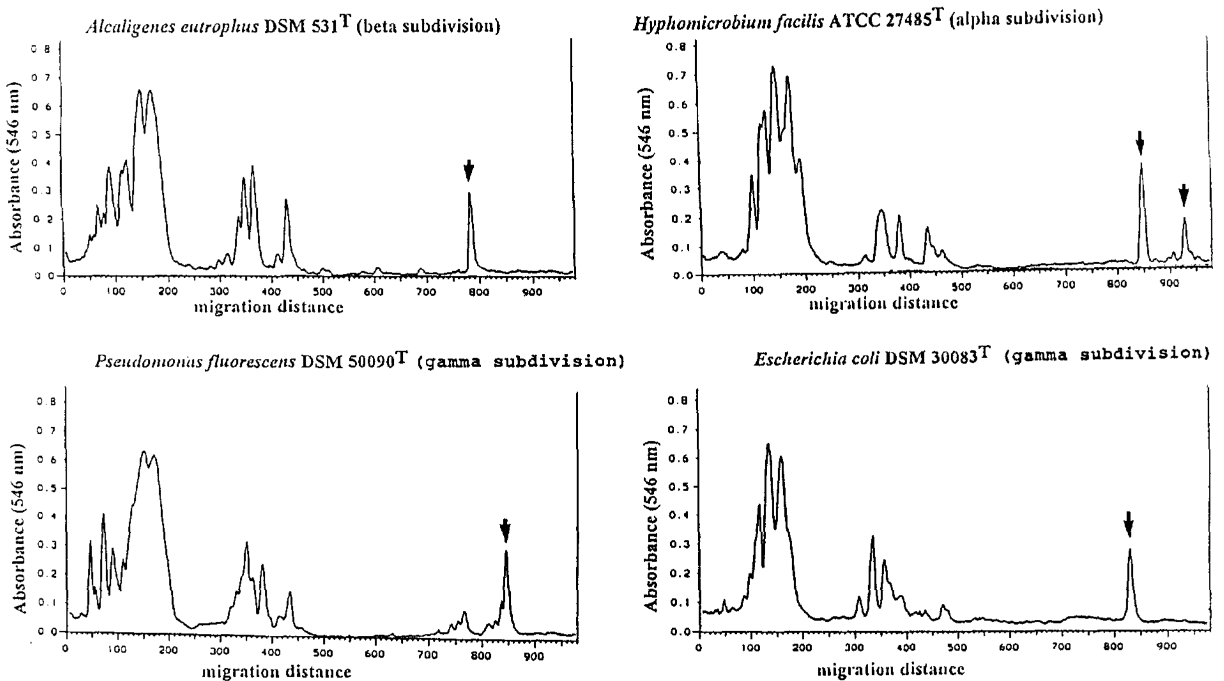


Fig. 8. Gel scans obtained from slab PAGE of LMM RNA fingerprints of bacteria from different genera. 5S rRNA peaks are arrowed.

than those produced by PAGE, indicating that CE is a more powerful means of distinguishing between individual strains.

Because CE using entangled polymers separates RNA molecules on the basis not only of size, but also other factors, such as spatial structure, this technique has potential advantages over PAGE in distinguishing similar strains that would have tRNA molecules of similar sizes. Additionally, the capacity of this technique to be automated lends itself to the analysis of a large number of strains, especially important in the field of ecology.

4. Conclusions

The use of CE with entangled polymer solutions represents an important technique for the rapid separation of bacterial LMM RNA producing a potentially useful genotypic fingerprint. These fingerprints may prove useful in the identification of single bacterial strains. CE generation of fingerprints offers a number of advantages over conventional PAGE techniques, including high sensitivity corresponding to very small sample size requirements (orders of magnitude less than conventional electrophoresis), good reproducibility, automated and easy handling, rapid run time (30 min) and on-line data collection. In addition to classical slab PAGE, CE using entangled polymer solutions can represent a convenient alternative method that uses non-denaturing separation conditions. The separation of the LMM RNA by CE is based on the size, the spatial structure, the changes in conformation of molecules and their interaction with the polymer network. Therefore, the profiles obtained by CE can give information additional to that obtained from conventional slab gel electrophoresis. In this context, the combined information from both methods could be of particular importance for future taxonomic studies.

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References

[1] R. Kuhn and S. Hoffstetter-Kuhn (Editors), Capillary Electrophoresis: Principles and Practice, Springer, Berlin, 1993, p. 251.
[2] D.N. Heiger, A.S. Cohen and B.L. Karger, J. Chromatogr., 516 (1990) 33.
[3] A. Paulus, E. Gassmann and M.J. Field, Electrophoresis, 11 (1990) 702.
[4] J.A. Luckey and L.M. Smith, Electrophoresis, 14 (1993) 492.
[5] P.D. Grossman and D.S. Soane, J. Chromatogr., 559 (1991) 257.
[6] M.H. Kleemiss, M. Gilges and G. Schomburg, Electrophoresis, 14 (1993) 515.
[7] B.R. McCord, J.M. Jung and E.A. Holleran, J. Liq. Chromatogr., 16 (1993) 1963.
[8] S.M. Clark and R.A. Mathies, Anal. Biochem., 215 (1993) 163.
[9] J. Cheng, T. Kasuga, K.R. Mitchelson, E.R.T. Lightly, N.D. Watson, W.J. Martin and D. Atkinson, J. Chromatogr. A, 677 (1994) 169.
[10] K.J. Ulfelder, H.E. Schwartz, J.M. Hall and F.J. Sunzeri, Anal. Biochem., 200 (1992) 260.
[11] F. Martin, D. Vairelles and B. Henrion, Anal. Biochem., 214 (1993) 182.
[12] E. Katsivela and M.G. Höfle, J. Chromatogr. A, 700 (1995) 125.
[13] M.G. Höfle, Arch. Microbiol., 153 (1990) 299.
[14] M.G. Höfle, Appl. Environ. Microbiol., 58 (1992) 3387.
[15] M.G. Höfle, J. Microbiol. Methods, 8 (1988) 235.
[16] D. Rickwood and B.D. Hames (Editors), Gel Electrophoesis of Nucleic Acids, IRL Press Oxford, 1982, p. 1.
[17] H.E. Schwartz, K.J. Ulfelder, F.J. Sunzeri, M.P. Busch and R.G. Brownlee J. Chromatogr., 559 (1991) 267.
[18] P.D. Grossman, J. Chromatogr. A, 663 (1994) 219.
[19] B.R. McCord, D.L. McClure and J.M. Jung, J. Chromatogr. A, 652 (1993) 75.
[20] M.S. Bello, P. de Besi, R. Rezzonico, P.G. Righetti and E. Casiraghi, Electrophoresis, 15 (1994) 623.
[21] Y.C. Bae and D. Soane, J. Chromatogr. A, 652 (1993) 17.