RESEARCH LETTER

Typing of bacteriophages by randomly amplified polymorphic DNA (RAPD)-PCR to assess genetic diversity

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Abstract

The recent boom in phage therapy and phage biocontrol requires the design of suitable cocktails of genetically different bacteriophages. The current methods for typing phages need significant quantities of purified DNA, may require *a priori* genetic information and are cost and time consuming. We have evaluated the randomly amplified polymorphic DNA (RAPD)-PCR technique to produce unique and reproducible band patterns from 26 different bacteriophages infecting *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Lactococcus lactis*, *Escherichia coli*, *Streptococcus thermophilus*, *Bacillus subtilis* and *Lactobacillus casei* bacterial strains. Initially, purified DNA and phage suspensions of seven selected phages were used as a template. The conditions that were found to be optimal 8 μM of 10-mer primers, 3 μM magnesium oxalacetate and 5% dimethyl sulfoxide. The RAPD genomic fingerprints using a phage titer suspension higher than 10^9 PFU mL^{-1} were highly reproducible. Clustering by the Pearson correlation coefficient and the unweighted pair group method with arithmetic averages clustering algorithm correlated largely with genetically different phages infecting the same bacterial species, although closely related phages with a similar DNA restriction pattern were indistinguishable. The results support the use of RAPD-PCR for quick typing of phage isolates and preliminary assessment of their genetic diversity bypassing tedious DNA purification protocols and previous knowledge of their sequence.

Introduction

Bacteriophages are ubiquitous in nature and found in all the habitats that their host bacteria colonize. It is now widely accepted that bacteriophages are the most abundant biological entities on Earth (10^{31} particles) (Brüssow & Kutter, 2005). They contribute largely to maintaining population densities and diversity of bacterial species, but also influence significantly biogeochemical and ecological processes including nutrient cycling, carbon flow and genetic transfer (Gill *et al.*, 2003; Thurber, 2009).

Classical bacteriophage taxonomy is based on their shape and size as well as their nucleic acid. Bacteriophages have been classified into 13 families; three of them (*Myoviridae*, *Siphoviridae* and *Podoviridae*) are members of the *Caudovirales* order that comprises about 96% of phages identified so far (5360 of 5568 reported to date, Ackermann, 2007). All these phages possess tail and double-stranded DNA.

The 500 bacteriophage genome sequences available at present in the NCBI phage database reveal the remarkable genetic diversity among phages, with genomes ranging from 15 up to 500 kb in size. Furthermore, bacteriophage genomes show a mosaic structure and each genome may be considered as a unique combination of modules whose size and rates of exchange vary considerably among the population. Nevertheless, despite the lack of similarity at the DNA level, phages encode proteins with significant sequence similarity, reflecting a common origin (Hendrix *et al.*, 1999). Recently, new phage classification schemes based on protein similarities have been developed for complementing the traditional classification (Lavigne *et al.*, 2008, 2009).

One of the main obstacles of phage biocontrol and phage therapy approaches is the narrow host range as a single

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Table 1. General features of bacteriophages, host bacteria and culturing conditions

Phage	Host bacteria	Bacteriophage family	Genome size (kbp)	Life cycle	Propagation conditions	References
vB_SepiS-phiIPLA4	*S. epidermidis* F12	*Siphoviridae*	39	Lytic	37 °C, shaking, TSB	D. Gutiérrez (unpublished data)
vB_SepiS-phiIPLA5	*S. epidermidis* F12	*Siphoviridae*	39	Lytic	37 °C, shaking, TSB	Gutiérrez *et al*. (2010)
vB_SepiS-phiIPLA6	*S. epidermidis* F12	*Siphoviridae*	38	Temperate	37 °C, shaking, TSB	Gutiérrez *et al*. (2010)
vB_SepiS-phiIPLA7	*S. epidermidis* F12	*Siphoviridae*	33	Temperate	37 °C, shaking, TSB	Gutiérrez *et al*. (2010)
vB_SauS-phiIPLA35	*S. aureus* Sa9	*Siphoviridae*	45.3	Lytic	37 °C, shaking, TSB	García *et al*. (2007)
ΦH5	*S. aureus* Sa9	*Siphoviridae*	42.5	Temperate	37 °C, shaking, TSB	García *et al*. (2007)
ΦC2	*L. lactis* MG1614	*Siphoviridae*	22.1	Lytic	30 °C, static, GM17	Lubbers *et al*. (1995)
Φ936	*L. lactis* IL1403	*Siphoviridae*	ND	Lytic	30 °C, static, GM17	Jarvis *et al*. (1991)
ΦP335	*L. lactis* F4.2	*Siphoviridae*	33.6	Lytic	30 °C, static, GM17	Braun *et al*. (1989)
ΦFIPLA-1	*S. thermophilus* St5	*Siphoviridae*	35.2	Lytic	42 °C, static, GLM17	Magadán (2007)
ΦFIPLA-3	*S. thermophilus* IPLA-10094	*Siphoviridae*	44.4	Lytic	42 °C, static, GLM17	Magadán (2007)
ΦFIPLA-120	*S. thermophilus* IPLA-10094	*Siphoviridae*	45.5	Lytic	42 °C, static, GLM17	Magadán (2007)
ΦFIPLA-122	*S. thermophilus* IPLA-10074	*Siphoviridae*	34.2	Lytic	42 °C, static, GLM17	Magadán (2007)
ΦFIPLA-126	*S. thermophilus* LMD9	*Siphoviridae*	35.2	Lytic	42 °C, static, GLM17	Magadán (2007)
ΦA2	*L. casei* ATCC393	*Siphoviridae*	43.4	Temperate	37 °C, static, MRS	Herrero *et al*. (1994)
ΦSPP1	*B. subtilis* 5B88G	*Siphoviridae*	44	Lytic	37 °C, static, 2 × YT	Riva *et al*. (1968)
ΦSOM1	*E. coli* WG5	*Siphoviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)
ΦSOM2	*E. coli* WG5	*Myoviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)
ΦSOM4	*E. coli* WG5	*Siphoviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)
ΦSOM7	*E. coli* WG5	*Siphoviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)
ΦSOM8	*E. coli* WG5	*Myoviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)
ΦSOM23	*E. coli* WG5	*Siphoviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)
ΦSOM28	*E. coli* WG5	*Siphoviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)
ΦSCH10	*E. coli* WG5	*Myoviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)
ΦP1	*E. coli* WG5	*Podoviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)
ΦX174	*E. coli* WG5	*Microviridae*	ND	Lytic	37 °C, shaking, 2 × YT	Muniesa *et al*. (1999)

ND, Not determined.

TSB, Tryptic soy broth (Scharlau Chemie S.A., Barcelona, Spain); GM17, M17 (Biokar, Beauvais, France) supplemented with glucose (0.5% p/v); GLM17, M17 supplemented with 0.5% glucose and 0.5% lactose; MRS, Man–Rogosa–Sharpe (Biokar); 2 × yeast extract and tryptone broth (YT), Sambrook *et al*. (1989).

phage may infect only specific strains. Thereby, the use of phage cocktails has been proposed (Sulakvelidze *et al*., 2001). However, assessment of the genetic diversity among a large collection of phage isolates would require effective propagation of each phage to isolate enough DNA for sequencing or analysis of DNA restriction patterns, which is time consuming and not always successful. Thus, a quick and reproducible approach would be very valuable to type new phages whose genome sequences are unknown. Pioneering work has made use of fluorescence-labelled restriction fragment length polymorphism (fRFLP) to address bacteriophage typing (Merabishvili *et al*., 2007).

Among other DNA-based approaches, random PCR amplifications of DNA segments using short primers of arbitrary nucleotide sequence have been used to generate specific profiles or genomic fingerprints that are used to compare the genotypic diversity among, for example, bacterial isolates (Johansson *et al*., 1995; Guglielmotti *et al*., 2006; Maiti *et al*., 2009), or whole bacterial communities (Franklin *et al*., 1999; Yang *et al*., 2000). Randomly amplified polymorphic DNA (RAPD)-PCR using purified DNA has also been used to assess the genetic diversity of vibriophages (Comeau *et al*., 2006; Shivu *et al*., 2007) and phages infecting *Escherichia coli* (Dini & de Urraza, 2010) and *Pseudomonas aeruginosa* (Li *et al*., 2010).

In this study, we have optimized a RAPD-PCR assay to evaluate whether reproducible patterns using phage lysates, instead of purified phage DNA, could be generated, as this would be more suitable for rapid screening of a high number of phage isolates.

Materials and methods

Bacteriophage propagation and purification

Twenty-six bacteriophages were used in this study (Table 1). Phage propagation was performed in broth by infecting early exponential bacterial cultures supplemented with 10 mM $Ca(NO_3)_2$ and 10 mM $MgSO_4$, at a multiplicity of infection of 1.0. Lysed bacterial cultures were centrifuged at 10 000 ***g***, the supernatants were filtered (0.45 μm, cellulose acetate membrane; VWR) and the phage titer was determined. Phage suspensions were dialyzed against distilled water for 1 h using 0.025-μm filters (MF-Millipore™ Membrane Filters; Millipore, Ireland) and stored at 4 °C.

Phage suspensions were also obtained from confluent lysis plaques on a solid medium. Appropriate phage dilutions were mixed with host bacteria in 0.7% top agar, poured on plates and incubated overnight. One milliliter of sterile-distilled water was added to plates and shaken for 1 h. The suspension was then centrifuged, and the supernatant was filtered and dialyzed as indicated above. Phage samples from both liquid and solid phage propagation were boiled for 10 min before the RAPD-PCR reaction.

Pure phage preparations were prepared by a CsCl continuous density gradient (Sambrook *et al.*, 1989). Briefly, 1 L of a bacterial lysate was centrifuged at 10 000 ***g***. Phages were recovered from the supernatant by 10% polyethylene glycol8000 and 0.5 M NaCl precipitation. After centrifugation (13 000 ***g***), phages were suspended in SM buffer (20 mg L^{-1} Tris-HCl, 10 mg L^{-1} $MgSO_4$, 10 mg L^{-1} $CaCl_2$, 100 mg L^{-1} NaCl, pH 7.5) containing RNase 40 μg mL^{-1}. Finally, phages were further purified by adding CsCl, followed by ultracentrifugation at 100 000 ***g*** at 4 °C for 20 h.

Phage DNA isolation

Phage DNA was extracted as described previously (García *et al.*, 2003) from 100 μL of purified phage stocks previously dialyzed against SM buffer.

Genomic fingerprinting by RAPD analysis

Random amplification of polymorphic DNA was carried out according to a modification of the method described previously (Johansson *et al.*, 1995). Primers OPL5 (5′-ACGCAGGCAC-3′), RAPD5 (5′-AACGCGCAAC-3′), P1 (5′-CCGCAGCCAA-3′) and P2 (5′-AACGGGCAGA-3′) were assayed at three different concentrations (1, 4 and 8 μM).

PCR reactions were performed using PureTaq™ Ready-To-Go™ PCR Beads (GE Healthcare, Munich, Germany) adding 10 ng of purified phage DNA or 10^7–10^8 plaque forming units (PFU) of phage suspensions. Reactions were supplemented with 3 mM magnesium oxalacetate and/or 5% v/v dimethyl sulfoxide (DMSO). PCR was performed in a thermocycler (Bio-Rad, Hercules) under the following thermal cycling conditions: four cycles at 94 °C for 45 s, 30 °C for 120 s and 72 °C for 60 s; 26 cycles at 94 °C for 5 s, 36 °C for 30 s and 72 °C for 30 s (the extension step was increased by 1 s for every new cycle); and a final step of 10 min at 75 °C.

Processing, comparison and reproducibility of RAPD patterns

DNA band patterns were obtained after gel electrophoresis (0.8% agarose gel) of the RAPD-PCR reaction products (15 μL). Gels were run for about 55 min at 100 V and stained in ethidium bromide (0.5 μg mL^{-1}) for 30 min. DNA molecular weight marker ('500 bp molecular ladder', Bio-Rad) was used as a standard. Gel images were processed using the software FINGERPRINTING II (Bio-Rad). The similarity matrix was calculated on the basis of the Pearson product–moment correlation coefficient, and its corresponding dendrogram was deduced using the unweighted pair group method with arithmetic averages [Struelens & the Members of the European Study Group on Epidemiological Markers (ESGEM), of the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), 1996]. Reproducibility was assessed by cluster analysis of triplicate reactions.

Results and discussion

RAPD-PCR analysis of purified phage DNA

RAPD-based methods do not require sequence information for PCR primer design. However, they are extremely dependent on laboratory conditions such as template DNA concentration, PCR and electrophoretic settings, among others (Ellsworth *et al.*, 1993). To establish a quick and useful RAPD-PCR protocol to type phages, phages infecting strains belonging to the same species (four *Staphylococcus epidermidis* phages), or different species within the same genus (two *Staphylococcus aureus* phages) or a different genus (one *Lactococcus lactis* phage) were selected to test several experimental conditions in order to generate reproducible RAPD patterns and gain a preliminary insight into the discrimination power of this approach.

The selected *S. epidermidis* phages belonged to the *Siphoviridae* family (morphotype B1) and their genome sequences were unknown. However, previous DNA restriction analysis revealed distinct patterns for the temperate phages ΦSepi-IPLA6 and ΦSepi-IPLA7, while the DNA restriction patterns of the lytic phages ΦSepi-IPLA4 and ΦSepi-IPLA5 (presumably virulent derivatives of ΦSepi-IPLA6) were very similar to each other (Gutiérrez *et al.*, 2010; and our unpublished data). The two phages infecting *S. aureus* ΦH5 and vB_SauS-phiIPLA35 (Φ35) belonged to morphotype B1 and morphotype B2, respectively, and their complete genome sequence was available (García *et al.*, 2007, 2009). Finally, the lytic *L. lactis* phage ΦC2 belonging to the morphotype B2 (Lubbers *et al.*, 1995) was chosen as representative of phages infecting a different genus within Gram-positive bacteria.

Initially, pure phage DNA (10 ng) was used as a template. Because RAPD-PCR reactions are considerably influenced by primers and their concentration (Johansson *et al.*, 1995), four primers (OPL5, RAPD5, P1 and P2) at three different concentrations (1, 4 and 8 μM) were tested. Furthermore, we tested whether the presence of magnesium oxalacetate and DMSO resulted in better defined band patterns. It has

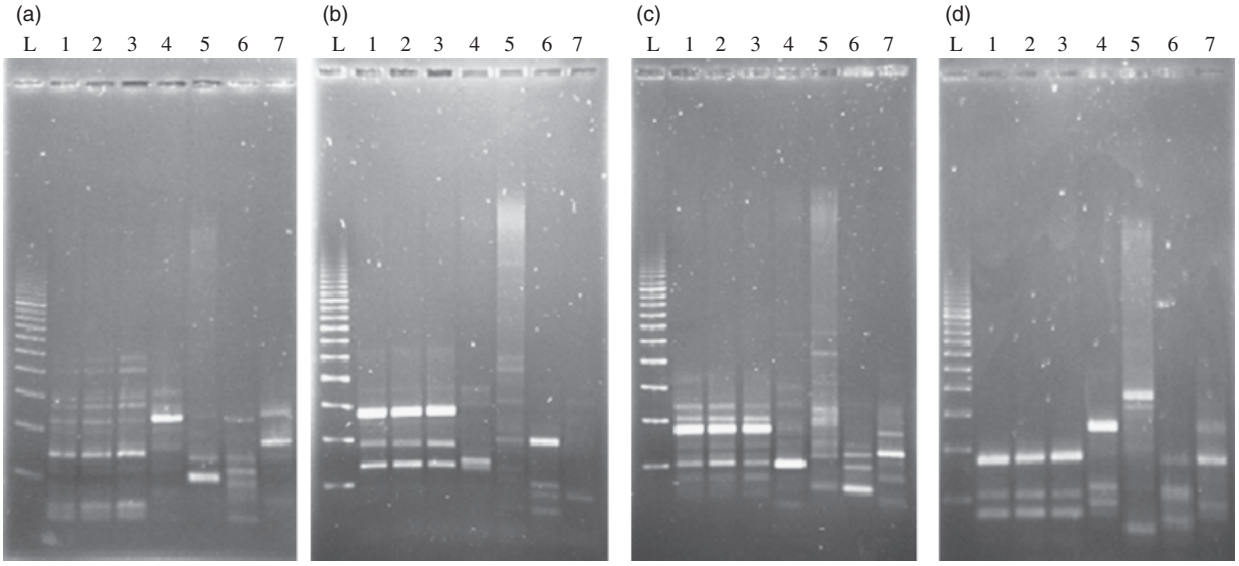

Fig. 1. RAPD band patterns obtained from seven different bacteriophages using pure DNA as a template and primers OPL5 (a), RAPD5 (b), P1 (c) and P2 (d) at 8 μM. Lane 1, vB_SepiS-phiIPLA4; lane 2, vB_SepiS-phiIPLA5; lane 3, vB_SepiS-phiIPLA6; lane 4, vB_SepiS-phiIPLA7; lane 5, vB_SauS-phiIPLA35; lane 6, ΦH5; and lane 7, ΦC2; lane L, 500 bp molecular ladder. Reactions contained magnesium oxalacetate (3 μM) and DMSO (5% v/v).

been described that Mg^{2+} ions form complexes with dNTPs, primers and template DNA, stimulating the action of DNA polymerase, and DMSO improves the DNA double-strand denaturalization and reduces secondary structures (Pomp & Medrano, 1991). Optimal results were obtained by the addition of 3 mM magnesium oxalacetate, 5% v/v DMSO and 8 μM primer concentration (Fig. 1). Lower primer concentrations produced less defined bands for primers OPL5 and RAPD5, and no amplification for primers P1 and P2 (data not shown). Similar observations were reported previously when typing *Lactobacillus plantarum* strains by RAPD-PCR in which the optimal primer concentration was also 8 μM (Johansson *et al.*, 1995).

As shown in Fig. 1, each primer generated distinct band patterns with amplicons ranging in size from approximately 500 bp to 12 kb. A total of 18 bands were observed for primer OPL5 (Fig. 1a), showing a greater discrimination among phages than the other primers that generated fewer (11–16) different bands (Fig. 1).

With the exception of *S. epidermidis* phages vB_SepiS-phiIPLA4, vB_SepiS-phiIPLA5 and vB_SepiS-phiIPLA6, which had shown a closely related DNA restriction pattern, the RAPD-PCR band profiles were unique for each phage (Fig. 1). It is worth noting that *L. lactis* phage ΦC2 generated a small number of bands with all the primers assayed (Fig. 1, lane 7). Its lower genome size (22 163 bp) could explain this result (see Table 1).

The genomic fingerprints resulting from the amplification of phage DNA samples performed on three separate days were compared to determine the RAPD-PCR reproducibility (Table 2). Each phage showed an identical band profile regardless of the assay date. Primers OLP5 and P2 provided high reproducibility values for genomic fingerprints and performed better than RAPD5 and P1. The low reproducibility of the later primers could be explained by the low number of amplification products obtained from phage ΦC2 with RAPD5 (see Fig. 1). Moreover, differences in the band intensity on phage ΦH5 DNA may have accounted for the low reproducibility of P1 (data not shown). No reproducible band intensities were likely due to nonspecific annealing between the primer and the DNA template as reported previously (Pérez *et al.*, 1998).

Table 2. Reproducibility, indicated as Pearson's product correlation coefficient, of the RAPD-PCR reactions performed with different primers and templates

	Primer			
Template	OPL5 (%)	RAPD5 (%)	P1 (%)	P2 (%)
Purified phage DNA	95	58	28	92
Phage suspensions (liquid propagation)	< 20	< 20	< 20	< 20
Phage suspensions (solid propagation)	> 90	25	> 90	60

RAPD-PCR analysis of phage suspensions

Phage suspensions were evaluated as source of DNA template to avoid the phage DNA purification step. Phage propagation in liquid and solid culture media yielded a titer of 10^7–10^8 and $> 10^9$ PFU mL^{-1}, respectively, for all

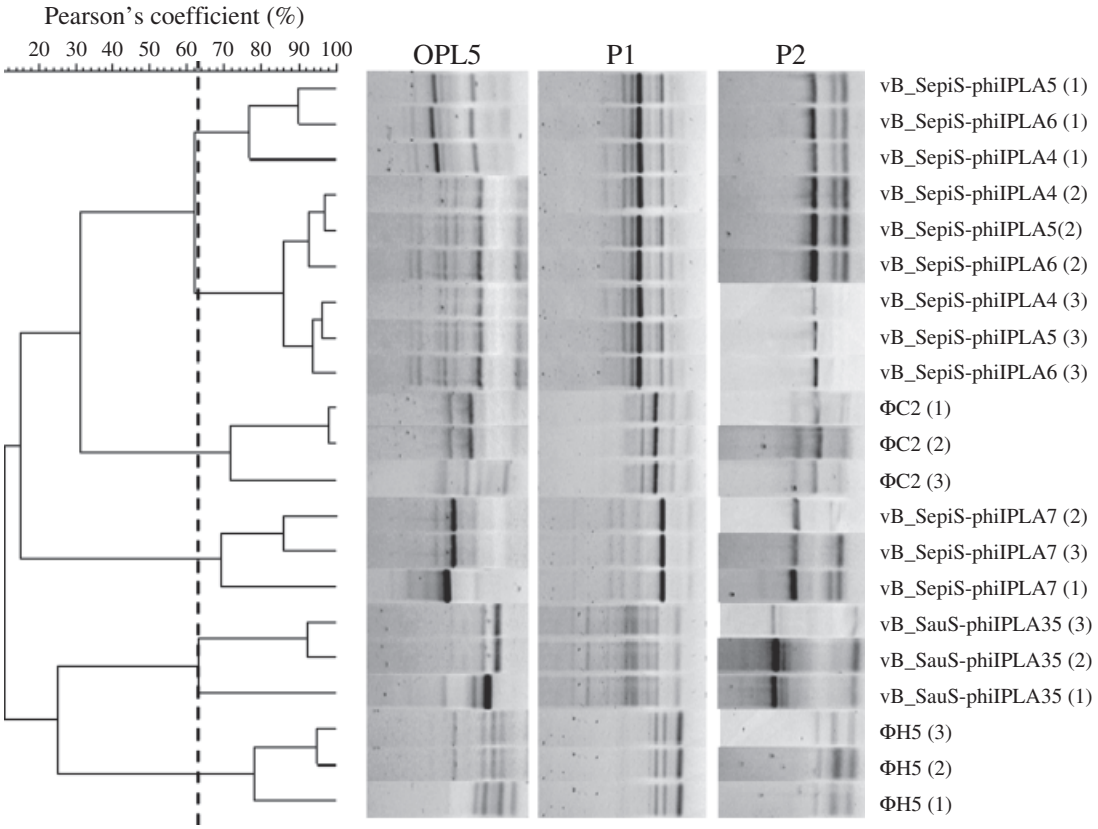

Fig. 2. Dendrogram obtained after the analysis of RAPD band patterns generated with different sources of DNA and combining the primers OPL5, P1 and P2. The DNA source used as a template is indicated in each lane: (1) DNA isolated from purified phage suspensions, (2) phage suspensions from a liquid medium, (3) phage suspensions from a solid medium. The similarity between samples was calculated on the basis of the Pearson product–moment correlation coefficient and its corresponding dendrogram was constructed using the unweighted pair group algorithm method with arithmetic averages. The identity level for genotypes discrimination is represented by a dashed line.

selected phages. To discard amplification from bacterial DNA, noninfected host bacterial cultures were processed under the same conditions as the phage lysates and used as a template in RAPD-PCR reactions. No amplification from host DNA was observed under the assay conditions (data not shown). Moreover, genomic fingerprints obtained using both phage lysates (from liquid and solid medium propagation) as a template were apparently similar to each other and to those obtained using pure DNA as a template (see Fig. 2).

The reproducibility of the assay using each template source with each single primer is shown in Table 2. In general, the RAPD profiles of phage suspensions from liquid propagation were poorly reproducible (< 20%) regardless of the primer used. By contrast, higher reproducibility values from phage suspensions obtained in a solid medium were recorded. Reproducibility seemed to be related to phage titer because suspensions from liquid propagation had 10–100 times less phages than those obtained from solid propagation ($\geq 10^9$ PFU mL^{-1}). We presume that the lower the phage titer, the lower DNA template is available for the PCR reaction, a factor that considerably influences the performance of the RAPD-PCR reaction (Ellsworth *et al.*, 1993). Therefore, the low reproducibility of phage suspensions from liquid propagation is likely linked to variations in the initial phage titer. Moreover, a phage titer higher than 10^9 PFU mL^{-1} seems to be required to obtain a suitable reproducibility when using phage suspensions as a DNA source.

Reproducibility analysis of RAPD-PCR combining type of template and primers OPL5, P1 and P2

A more detailed analysis was carried out comparing the genomic fingerprints generated from the three phage DNA sources with all three OPL5, P1 and P2 primers. RAPD5 was discarded due to the low reproducibility values obtained in the different assays. As shown in Fig. 2, the band patterns obtained from the different DNA templates clustered each phage together. As anticipated, the sensitivity of the RAPD-PCR assay was not enough to resolve the very close related *S. epidermidis* phages vB_SepiS-phiIPLA4, vB_SepiS-phiIPLA5 and vB_SepiS-phiIPLA6. Still, our results support the

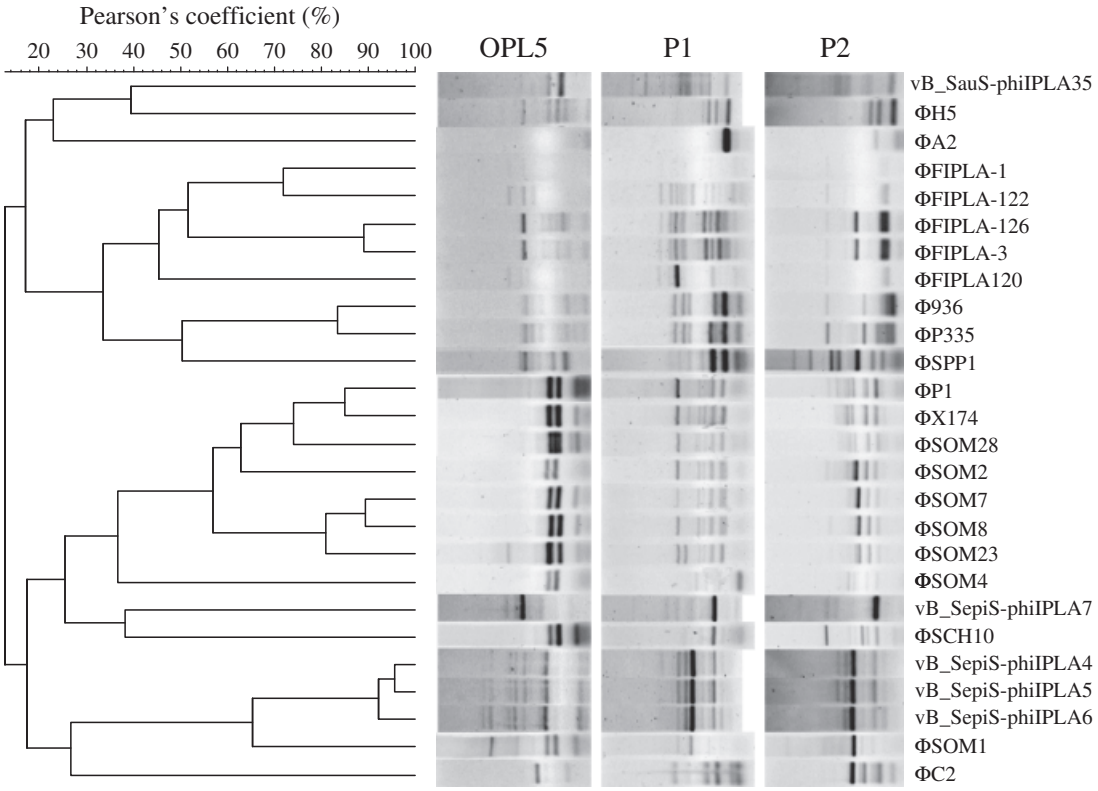

Fig. 3. Cluster analysis of the RAPD band patterns obtained from 26 different bacteriophages using the primers OPL5, P1 and P2. The similarity between samples was calculated on the basis of the Pearson product–moment correlation coefficient and its corresponding dendrogram was constructed using the unweighted pair group algorithm method with arithmetic averages.

use of sequence-specific 10-mer primers to reproducibly produce an adequate number of bands for the analysis of small genomes such as viruses. This is in accordance with previous reports showing that nondegenerate and degenerate 10-mer primers can produce robust band patterns for RAPD fingerprinting analysis (Comeau *et al.*, 2004; Winget & Wommack, 2008). In addition, pooling RAPD band patterns resulting from, at least, two different primers allows greater sensitivity.

Validation of RAPD-PCR to type genetically diverse bacteriophages

According to our results, phage suspensions are also suitable to generate reproducible RAPD profiles, bypassing the need for isolating DNA. Consequently, RAPD-PCR could be a cost-effective and time-saving technique to assess the genetic diversity among phages. To validate its discriminatory power further, the RAPD-PCR assay was performed on a wide group of 26 phages infecting both Gram-positive and Gram-negative bacteria ranging from 33% to 50% in their G+C content. These phages belong to four different families (*Siphoviridae*, *Podoviridae*, *Myoviridae* and *Microviridae*). Phages infecting *L. lactis*, *Streptococcus thermophilus*, *Lactobacillus casei*, *Bacillus subtilis* and *E. coli* were used in the validation assay (Table 1). Genomic fingerprints were generated from phage suspensions after solid medium propagation using primers OPL5, P1 and P2 and the combined patterns were analyzed (Fig. 3).

The RAPD profiles were distinct for each phage and revealed the existence of four main clusters. These clusters matched largely with the bacterial species and most of the phages infecting the same bacterial species were clustered together, with few exceptions. Phages infecting *S. thermophilus* showed closed, but distinguishable patterns and slightly related to Φ936, ΦP335 and ΦSPP1. *Escherichia coli* phages also clustered together, except ΦSOM1. Finally, *S. epidermidis* phages were also grouped, vB_SepiS-phiIPLA7 being the exception.

This clustering was not surprising because of the phylogenetic relations among phages. As it has been described previously, phages infecting distantly related bacterial hosts typically share little or no nucleotide sequence similarity, while phages infecting a specific bacterial host are more similar (Hatfull, 2008). Moreover, module exchanging could be the reason why phages vB_SepiS-phiIPLA7, ΦC2 and ΦSOM1 were grouped into a different cluster than the other phages infecting the same bacterial host.

Phage morphology did not correlate with the RAPD-PCR clustering as phages belonging to different morphological families were grouped together. This is the case of ΦX174 (*Microviridae*), ΦP1 (*Podoviridae*), ΦSOM8 and ΦSOM2 (*Myoviridae*), which were clustered with the rest of the phages belonging to the *Siphoviridae* family. The classification in families is mostly based on virion morphology and nucleic acid type, and bacteriophages belonging to different families may have similar DNA sequences (Ackermann, 2003). Thereby, similar RAPD-PCR profiles can be found among families. A similar discrepancy has already been reported when using fRFLP for bacteriophage typing (Merabishvili *et al.*, 2007).

It remains to be confirmed whether RAPD typing using phage lysates is also a feasible technique when using phages infecting high G+C bacterial hosts as those were not included in this study. However, based on the use of DMSO in the reaction buffer and the availability of enhanced DNA polymerases and buffers active on high G+C DNA templates, it is reasonable to speculate that this approach may also be useful.

Conclusions

RAPD-PCR on phage suspensions is a suitable approach to quickly assess the genetic diversity among newly isolated bacteriophages infecting the same species while circumventing the need for DNA extraction and purification. Using this assay, genomic fingerprints from different phages infecting *Staphylococcus*, *Bacillus*, *E. coli*, *Lactococcus* and *Streptococcus* were distinct and showed variations in the number of bands, fragment size and intensity.

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Authors' contribution

P.G. and B.M. contributed equally to this work.

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