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1 **Title:** Typing of bacteriophages by randomly amplified polymorphic DNA (RAPD-
2 PCR) to assess genetic diversity
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21 **Running title**: Bacteriophage typing by RAPD-PCR.
22
23 **Keywords**: bacteriophage, typing, RAPD-PCR
24
25

26 **Abstract**

27

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

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49

50 **Introduction**

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

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

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

71 One of the main obstacles of phage biocontrol and phage therapy approaches is
72 the narrow host range as a single phage may infect only specific strains. Thereby, the
73 use of phage cocktails has been proposed (Sulakvelidze et al., 2001). However, to assess
74 the genetic diversity among a large collection of phage isolates would require effective

75 propagation of each phage to isolate enough DNA for sequencing or analysis of DNA
76 restriction patters which is time-consuming and not always successful. Thus, a quick
77 and reproducible approach would be very valuable to type new phages whose genome
78 sequences are unknown. Pioneering work has made use of fluorescence labelled
79 restriction fragment length polymorphism (fRFLP) to address bacteriophage typing
80 (Merabishvili et al., 2007).

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

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

93

94 **Material and Methods**

95 **Bacteriophage propagation and purification**

96 Twenty six bacteriophages were used in this study (Table 1). Phage propagation was
97 performed in broth by infecting early exponential bacterial cultures supplemented with
98 10 mM $Ca(NO_3)_2$ and 10 mM $MgSO_4$, at a multiplicity of infection (MOI) of 1.0. Lysed
99 bacterial cultures were centrifuged at 10,000 × g, the supernatants filtered (0.45 µm,
100 cellulose acetate membrane, VWR, USA) and the phage titer determined. Phage
101 suspensions were dialyzed against distilled water for 1 h using 0.025 µm filters (MF-
102 Millipore™ Membrane Filters. Millipore, Ireland) and stored at 4 °C.

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

109 Pure phages preparations were prepared by a CsCl continuous density gradient
110 (Sambrook et al., 1989). Briefly, 1 L of a bacterial lysate was centrifuged at 10,000 × g.
111 Phages were recovered from the supernatant by 10% polyethylene glycol (PEG) 8000
112 and 0.5 M NaCl precipitation. After centrifugation (13000 × g), phages were suspended
113 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}
114 NaCl, pH 7.5) containing RNAse 40 µg mL^{-1}. Finally, phages were further purified by
115 adding CsCl, followed by ultracentrifugation at 100,000 × g at 4 ºC for 20 h.

116

117 **Phage DNA isolation**

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

120

121 **Genomic fingerprinting by RAPD analysis**

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

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

135

136 **Processing, comparison, and reproducibility of RAPD patterns**

137 DNA band patterns were obtained after gel electrophoresis (0.8% agarose gel) of the
138 RAPD-PCR reaction products (15 µl). Gels were run for about 55 min at 100 V and
139 stained in ethidium bromide (0.5 µg mL^{-1}) for 30 min. DNA molecular weight marker
140 (‘500 bp molecular ladder’, Bio-Rad, Hercules, USA) was used as standard. Gel images
141 were processed with the software Fingerprinting II (Bio-Rad, Hercules, USA). The

142 similarity matrix was calculated on the basis of the Pearson product moment correlation

143 coefficient, and its corresponding dendrogram was deduced using the unweighted-pair-

144 group method with arithmetic averages (UPGMA) (Struelens et al., 1996).

145 Reproducibility was assessed by cluster analysis of triplicate reactions.

146

147

148 **Results and discussion**

149 **RAPD-PCR analysis of purified phage DNA**

150 RAPD based methods do not require sequence information for PCR primer design.
151 However, they are extremely dependent on laboratory conditions such as template DNA
152 concentration, PCR and electrophoretic settings among others (Ellsworth et al., 1993).
153 To establish a quick and useful RAPD-PCR protocol to type phages, phages infecting
154 strains belonging to the same species (4 *Staphylococcus epidermidis* phages), or
155 different species within the same genus (2 *Staphylococcus aureus* phages) or a different
156 genus (1 *Lactococcus lactis* phage) were selected to test several experimental conditions
157 in order to generate reproducible RAPD patterns and get a preliminary insight into the
158 discrimination power of this approach.

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

170 Initially, pure phage DNA (10 ng) was used as template. Since RAPD-PCR
171 reactions are greatly influenced by primers and their concentration (Johansson et al.,
172 1995), four primers (OPL5, RAPD5, P1 and P2) at three different concentrations (1 μM,

173 4 μM and 8 μM) were tested. Furthermore, we tested if the presence of magnesium
174 oxalacetate and DMSO resulted in better defined band patterns. It has been described
175 that Mg^{2+} ions form complexes with dNTPs, primers and template DNA, stimulating the
176 action of DNA polymerase and DMSO improves the DNA double strand
177 denaturalization and reduces secondary structures (Pomp & Medrano, 1991). Optimal
178 results were obtained by the addition of 3 mM magnesium oxalacetate, 5% v/v DMSO
179 and 8 μM primer concentration (Fig. 1). Lower primer concentrations produced less
180 defined bands for primers OPL5 and RAPD5, and no amplification for primers P1 and
181 P2 (data not shown). Similar observations were previously reported when typing
182 *Lactobacillus plantarum* strains by RAPD-PCR in which the optimal primer
183 concentration was also 8 μM (Johansson et al., 1995).

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

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

194 The genomic fingerprints resulting from amplification of phage DNA samples
195 performed on three separate days were compared to determine the RAPD-PCR
196 reproducibility (Table 2). Each phage showed identical band profile regardless of the
197 assay date. Primers OLP5 and P2 provided high reproducibility values for genomic

198 fingerprints and performed better than RAPD5 and P1. The low reproducibility of the
199 later primers could be explained by the low number of amplification products obtained
200 from phage ΦC2 with RAPD5 (see Fig. 1). Moreover, differences in band intensity on
201 phage ΦH5 DNA may have accounted for the low reproducibility of P1 (data not
202 shown). No reproducible band intensities were likely due to non-specific annealing
203 between the primer and the DNA template as previously reported (Pérez et al., 1998).
204

205 **RAPD-PCR analysis of phage suspensions**

206 Phage suspensions were evaluated as source of DNA template to avoid the phage DNA
207 purification step. Phage propagation in liquid and solid culture media yielded a titer of
208 10^7-10^8 pfu/ml and > 10^9 pfu/ml, respectively, for all selected phages. To discard
209 amplification from bacterial DNA, non-infected host bacterial cultures were processed
210 in the same conditions as the phage lysates and used as template in RAPD-PCR
211 reactions. No amplification from host DNA was observed in the assay conditions (data
212 not shown). Moreover, genomic fingerprints obtained using both phage lysates (from
213 liquid and solid medium propagation) as template were apparently similar to each other
214 and to those obtained using pure DNA as template (see Fig. 2).

215 The reproducibility of the assay using each template source with each single
216 primer is shown in Table 2. In general, RAPD profiles of phage suspensions from liquid
217 propagation were poorly reproducible (<20%) regardless of the primer used. By
218 contrast, higher reproducibility values from phage suspensions obtained in solid
219 medium were recorded. Reproducibility seemed to be related to phage titer since
220 suspensions from liquid propagation had 10 to 100 times less phages than those
221 obtained from solid propagation ($\geq 10^9$ pfu/ml). We presume that the lower the phage
222 titer, the lower DNA template is available for the PCR reaction, a factor which greatly

223 influences the performance of the RAPD-PCR reaction (Ellsworth et al., 1993).
224 Therefore, the low reproducibility of phage suspensions from liquid propagation is
225 likely linked to variations in the initial phage titer. Moreover, a phage titer higher than
226 10^9 pfu/ml seems to be required to obtain a suitable reproducibility when using phage
227 suspensions as DNA source.
228

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

231 A more detailed analysis was carried out comparing the genomic fingerprints generated
232 from the three phage DNA sources with all three OPL5, P1 and P2 primers. RAPD5
233 was discarded due to the low reproducibility values obtained in the different assays. As
234 shown in Fig. 2, the band patterns obtained from the different DNA templates clustered
235 each phage together. As anticipated, the sensitivity of the RAPD-PCR assay was not
236 enough to resolve the very close related *S. epidermidis* phages vB_SepiS-phiIPLA4,
237 vB_SepiS-phiIPLA5, vB_SepiS-phiIPLA6. Still, our results support the use of
238 sequence-specific 10-mer primers to reproducibly produce an adequate number of bands
239 for analysis of small genomes such as viruses. This is in accordance with previous
240 reports showing that non degenerate and degenerate 10-mer primers can produce robust
241 band patterns for RAPD fingerprinting analysis (Comeau et al. 2004; Winget &
242 Wommack, 2008). In addition, pooling RAPD band patterns resulting from, at least, two
243 different primers allow greater sensitivity.
244

245 **Validation of RAPD-PCR to type genetically diverse bacteriophages**

246 According to our results, phage suspensions are also suitable to generate reproducible
247 RAPD profiles bypassing the need of isolating DNA. Consequently, RAPD-PCR could

248 be a cost-effective and time-saving technique to assess the genetic diversity among
249 phages. To validate further its discriminatory power, the RAPD-PCR assay was
250 performed on a wide group of 26 phages infecting both Gram-positive and Gram-
251 negative bacteria ranging from 33% to 50% in their G+C content. These phages belong
252 to four different families (*Siphoviridae, Podoviridae, Myoviridae* and *Microviridae*).
253 Phages infecting *L. lactis*, *S. thermophilus*, *Lb. casei*, *B. subtilis* and *E. coli* were used in
254 the validation assay (Table 1). Genomic fingerprints were generated from phage
255 suspensions after solid medium propagation by using primers OPL5, P1 and P2 and the
256 combined patterns were analysed (Fig. 3).
257 RAPD profiles were distinct for each phage and revealed the existence of four
258 main clusters. These clusters matched largely with the bacterial species and most of the
259 phages infecting the same bacterial species were clustered together, with few
260 exceptions. Phages infecting *S. thermophilus* showed closed but distinguishable patterns
261 and slightly related to Φ936, ΦP335 and ΦSPP1. *E. coli* phages also clustered together
262 except ΦSOM1. Finally, *S. epidermidis* phages were also grouped, being vB_SepiS-
263 phiIPLA7 the exception.
264 This clustering was not surprising because of the phylogenetic relations among
265 phages. As it has been previously described, phages infecting distantly related bacterial
266 hosts typically share little or no nucleotide sequence similarity, while phages infecting a
267 specific bacterial host are more similar (Hatfull, 2008). Moreover, module exchanging
268 could be the reason why phages vB_SepiS-phiIPLA7, ΦC2 and ΦSOM1 were grouped
269 in a different cluster than the other phages infecting the same bacterial host.
270 Phage morphology did not correlate with the RAPD-PCR clustering as phages
271 belonging to different morphological families were grouped together. This is the case of
272 ΦX174 (*Microviridae*), ΦP1 (*Podoviridae*), ΦSOM8 and ΦSOM2 (*Myoviridae*) which

273 were clustered with the rest of phages belonging to the *Siphoviridae* family. The
274 classification in families is mostly based on virion morphology and nucleic acid type,
275 and bacteriophages belonging to different families may have similar DNA sequences
276 (Ackerman, 2003). Thereby, similar RAPD-PCR profiles can be found among families.
277 A similar discrepancy has been already reported when using fRFLP for bacteriophage
278 typing (Merabishvili et al., 2007).
279 It remains to be confirmed if RAPD typing using phage lysates is also a feasible
280 technique when using phages infecting high G+C bacterial hosts as those were not
281 included in this study. However, based on the use of DMSO in the reaction buffer and
282 the availability of enhanced DNA polymerases and buffers active on high G+C DNA
283 templates, it is reasonable to speculate that this approach may be also useful.
284

285 **Conclusions**

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

292

293 **Acknowledgments**

294 This work was supported by grants AGL2009-13144-C02-01 from the Ministry of

295 Education of Spain, IB08-052 from FICYT (Regional Government of Asturias) and

296 PIE200970I090 (CSIC, Spain). Thanks are given to M. Muniesa, M. A. Álvarez, J. E.

297 Suárez, and S. Ayora for kindly providing *E. coli, S. thermophilus, L. lactis, Lb. casei*

298 *and B. subtilis* bacteriophages used in this study.

299
300

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Figure captions

Fig. 1. RAPD band patterns obtained from seven different bacteriophages using pure DNA as 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).

Fig. 2. Dendrogram obtained after 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 liquid medium, (3) Phage suspensions from 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.

Fig. 3. Cluster analysis of 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.

Table 1.

General features of bacteriophages, host bacteria and culturing conditions.

Phage	Host bacteria	Bacteriophage family	Genome size (kbp)[a]	Life cycle	Propagation conditions[b]	Reference
vB_SepiS-phiIPLA4	*S. epidermidis* F12	*Siphoviridae*	39	lytic	37°C, shaking, TSB	Gutiérrez unpublished
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	42ºC, static, GLM17	Magadán, 2007
ΦFIPLA-3	*S. thermophilus* IPLA-10094	*Siphoviridae*	44.4	lytic	42ºC, static, GLM17	Magadán, 2007
ΦFIPLA-120	*S. thermophilus* IPLA-10094	*Siphoviridae*	45.5	lytic	42ºC, static, GLM17	Magadán, 2007
ΦFIPLA-122	*S. thermophilus* IPLA-10074	*Siphoviridae*	34.2	lytic	42ºC, static, GLM17	Magadán, 2007
ΦFIPLA-126	*S. thermophilus* LMD9	*Siphoviridae*	35.2	lytic	42ºC, static, GLM17	Magadán, 2007
ΦA2	*Lb. casei* ATCC393	*Siphoviridae*	43.4	temperate	37ºC, static, MRS	Herrero et al., 1994
ΦSPP1	*B. subtilis* 5B88G	*Siphoviridae*	44	lytic	37ºC, static, 2xYT	Riva et al., 1968
ΦSOM1	*E. coli* WG5	*Siphoviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999
ΦSOM2	*E. coli* WG5	*Myoviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999
ΦSOM4	*E. coli* WG5	*Siphoviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999
ΦSOM7	*E. coli* WG5	*Siphoviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999
ΦSOM8	*E. coli* WG5	*Myoviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999

ΦSOM23	*E. coli* WG5	*Siphoviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999
ΦSOM28	*E. coli* WG5	*Siphoviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999
ΦSCH10	*E. coli* WG5	*Myoviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999
ΦP1	*E. coli* WG5	*Podoviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999
ΦX174	*E. coli* WG5	*Microviridae*	ND	lytic	37°C, shaking, 2xYT	Muniesa et al., 1999

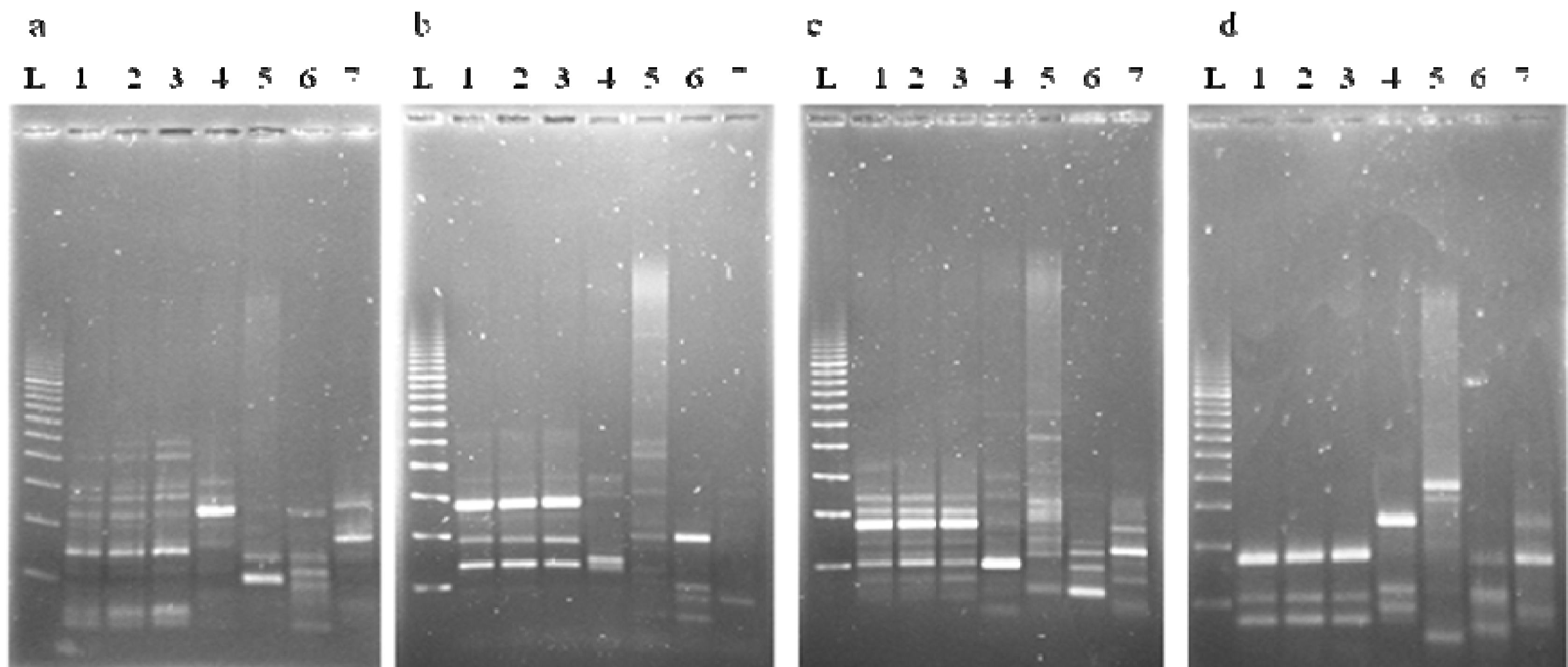
[a]ND: not determined.

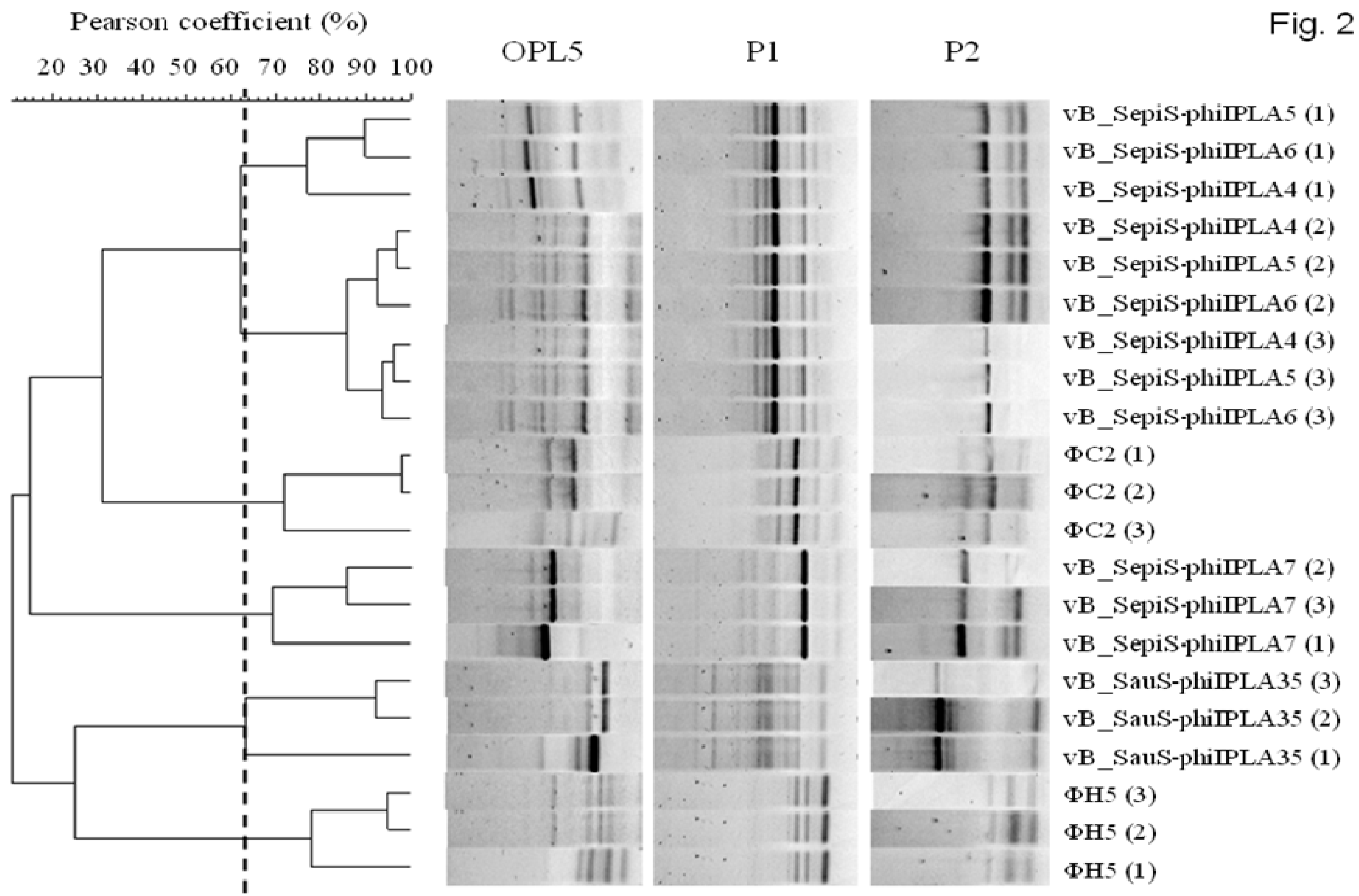
[b]TSB: Tryptic soy broth (Scharlau Chemie, S.A. Barcelona, Spain); GM17: M17 (Biokar, Beauvais, France) supplemented with glucose (0.5% p/v); GLM17: M17 supplemented with 0.5% glucose and 0.5% lactose; MRS: Man-Rogosa-Sharpe (Biokar); 2xYT: (Sambrook et al., 1989).

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

Template	Primer			
	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%

Fig. 1




Pearson coefficient (%)
20 30 40 50 60 70 80 90 100
OPL5
P1
P2
Fig. 2
vB_SepiS-phiIPLA5 (1)
vB_SepiS-phiIPLA6 (1)
vB_SepiS-phiIPLA4 (1)
vB_SepiS-phiIPLA4 (2)
vB_SepiS-phiIPLA5 (2)
vB_SepiS-phiIPLA6 (2)
vB_SepiS-phiIPLA4 (3)
vB_SepiS-phiIPLA5 (3)
vB_SepiS-phiIPLA6 (3)
ΦC2 (1)
ΦC2 (2)
ΦC2 (3)
vB_SepiS-phiIPLA7 (2)
vB_SepiS-phiIPLA7 (3)
vB_SepiS-phiIPLA7 (1)
vB_SauS-phiIPLA35 (3)
vB_SauS-phiIPLA35 (2)
vB_SauS-phiIPLA35 (1)
ΦH5 (3)
ΦH5 (2)
ΦH5 (1)

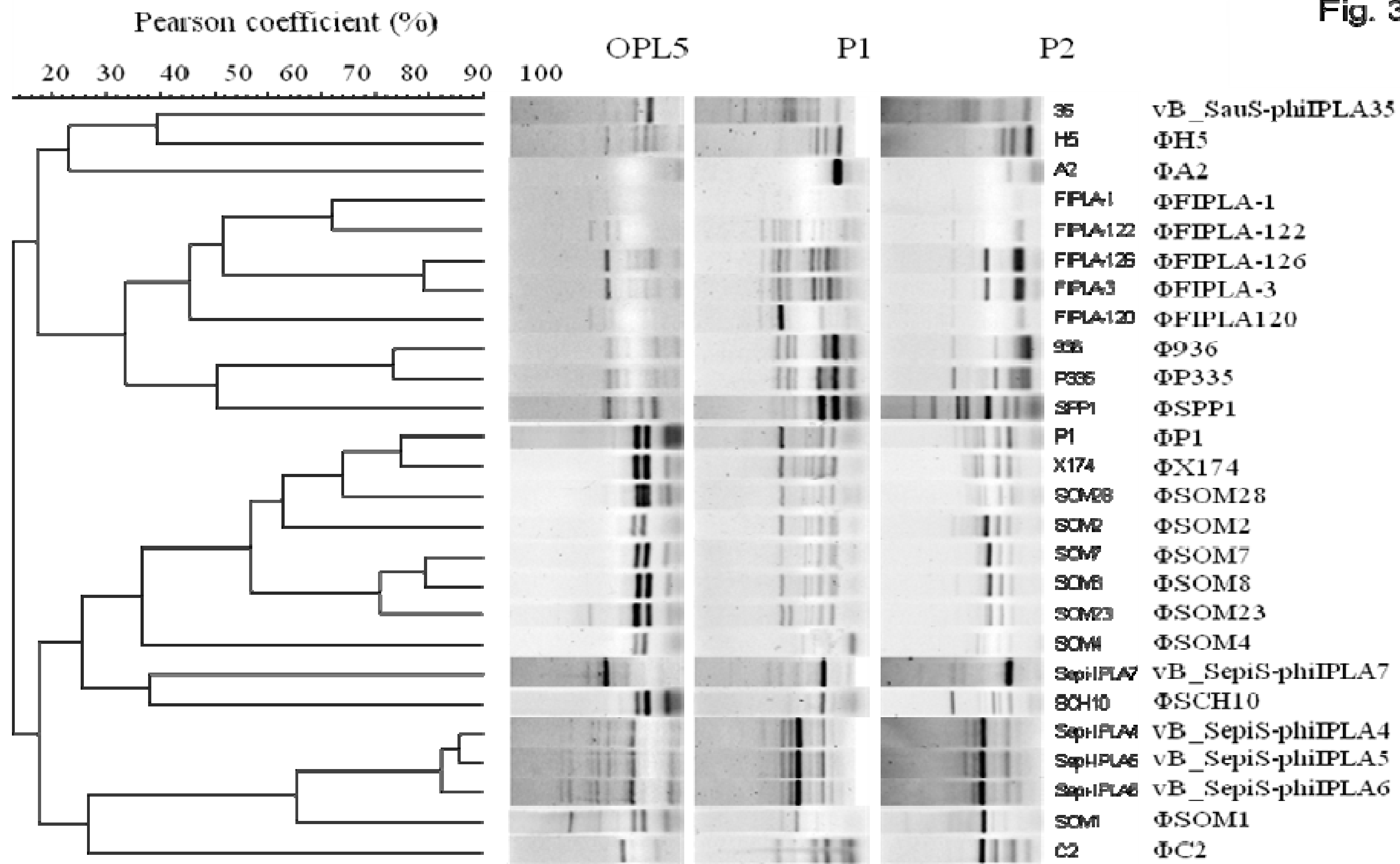


Fig. 3
Pearson coefficient (%)
20
30
40
50
60
70
80
90
100
OPL5
P1
P2
vB_SauS-phiIPLA35
ΦH5
ΦA2
ΦFIPLA-1
ΦFIPLA-122
ΦFIPLA-126
ΦFIPLA-3
ΦFIPLA120
Φ936
ΦP335
ΦSPP1
ΦP1
ΦX174
ΦSOM28
ΦSOM2
ΦSOM7
ΦSOM8
ΦSOM23
ΦSOM4
vB_SepiS-phiIPLA7
ΦSCH10
vB_SepiS-phiIPLA4
vB_SepiS-phiIPLA5
vB_SepiS-phiIPLA6
ΦSOM1
ΦC2