Comparison of genomes of closely related phages ø29, ø15 and PZA using a rapid method of parallel physical mapping

Zdeněk Hostomský, Václav Pačes and Stanislav Zadražil

Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Flemingovo nám.2, 166 37 Prague 6, Czechoslovakia

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The DNAs of phages ø29, ø15 and PZA of *Bacillus subtilis* were analysed with restriction enzymes *Eco*RI, *Hpa*I and *Hin*dIII. A method was used which permits parallel physical mapping of all three phages, from both ends of their linear genomes. The method is based on transfer of partially digested DNA to DBM paper and sequential hybridization with labelled terminal fragments. It follows from the comparison of the physical maps that phages ø29, ø15 and PZA are closely related and that they probably have arisen from a common ancestor by accumulation of point mutations.

Bacillus subtilis *phage* *DBM paper hybridization* *Restriction analysis* *Physical mapping*

1. INTRODUCTION

Bacteriophage ϕ29, which infects *Bacillus subtilis*, is well characterized genetically [1]. Similar to ϕ29 are less studied phages ϕ15 [2] and PZA [3]. The common characteristic of all these phages is that they contain a linear double-stranded DNA of about 19 kb with terminal proteins covalently linked to their 5′-ends. The terminal protein is involved in the initiation of replication of the ϕ29 linear genome [4,5].

Phages ϕ29, ϕ15 and PZA are indistinguishable by morphology. Some differences, however, have been observed in restriction patterns of their DNA [6] and in the host range [7]. To characterize further the differences among these phages at the level of genomic DNA, a physical mapping strategy was designed which permits parallel restriction analysis of several homologous DNAs.

2. MATERIALS AND METHODS

The phage DNA was isolated according to [8]. Restriction enzymes were from Miles (*Hpa*I) and New England Biolabs (*Eco*RI, *Hin*dIII).

For mapping the phages DNAs were partially digested with restriction enzymes, separated by electrophoresis in agarose gel and blotted to DBM paper [9]. The blot was hybridized with a nick-translated terminal fragment. After autoradiography the radioactivity was washed away by incubation for 1–3 h at 40°C in 99% formamide and the blot was hybridized again to the nick-translated terminal fragment isolated from the opposite end of the phage DNA. From autoradiograms the relative positions of restriction sites in all 3 phages can be derived.

Abbreviations: DBM, diazobenzyloxymethyl; bp, base pairs; kb, kilobase pairs

3. RESULTS

The ordering of restriction fragments in physical maps of phages ϕ29, ϕ15 and PZA, as shown in fig.3, is based on the following sets of data:

(1) Number and length of restriction fragments as derived from electrophoreses of total digests (fig.1). (Potential fragments of length less than 70 bp have been neglected.)

(2) Relative positions of restriction sites as derived from autoradiograms after hybridization of

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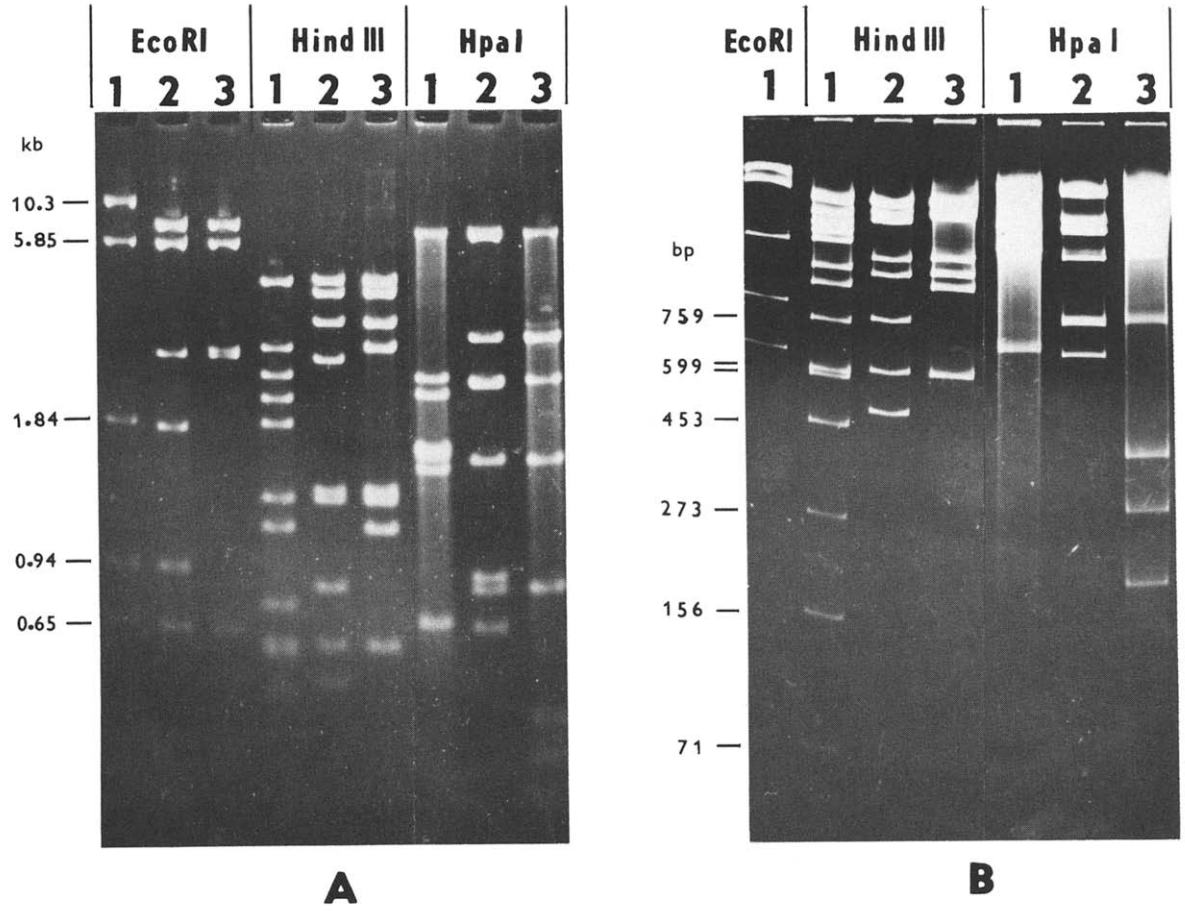

Fig.1. Restriction analysis of DNAs of phages ϕ29 (1), ϕ15 (2) and PZA (3). The same digests by restriction endonucleases *Eco*RI, *Hin*dIII and *Hpa*I were analysed in 1.3% agarose gel (A, focusing on larger fragments) and in 5% polyacrylamide gel (B, focusing on smaller fragments). The numbers on the left represent fragment sizes in the *Eco*RI digest of ϕ29 DNA (A) and in the *Hin*dIII digest (fragments H–N) of ϕ29 DNA (B).

partial digests of phage DNAs with radioactively labelled terminal fragments (fig.2).

The known restriction fragments and their ordering in the physical maps of phage ϕ29 [10] served as internal standard.

4. DISCUSSION

The method of physical mapping used here is based on a principle similar to that described by Smith and Birnstiel [11], i.e. on radioactive visualization of fragments containing terminal region of the mapped DNA in partial digests by restriction enzymes. However, our approach may have some advantages:

While the procedure of Smith and Birnstiel starts with partial digestion of a DNA fragment labelled at one end (usually obtained by end-labelling, cleavage in asymmetrically positioned restriction site, and isolation of labelled fragment), ours commences with partial digestion of unlabelled whole-length DNA. The sequential hybridization with isolated nick-translated terminal fragments permits the positions of restriction sites to be read twice in one digest, from both ends of the DNA along the whole molecule. The distal restriction sites become the closer ones when

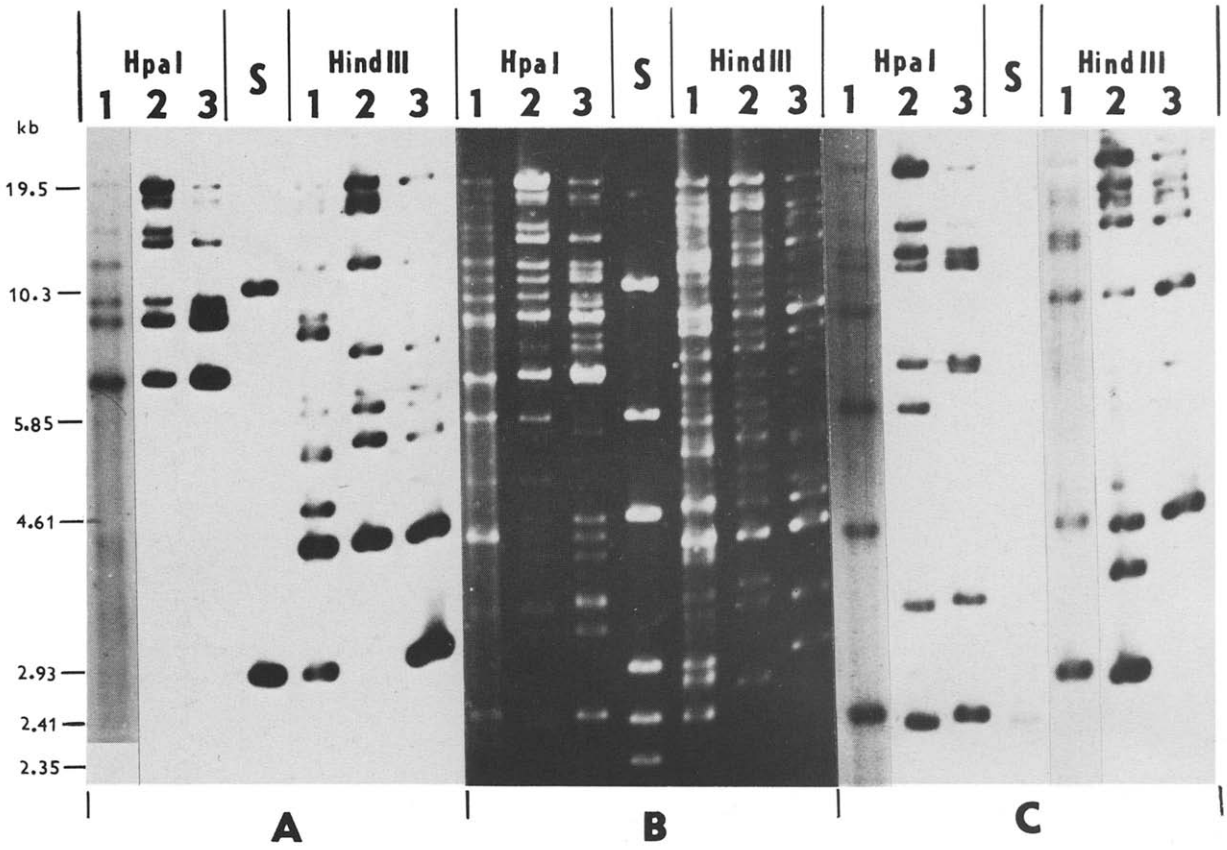

Fig.2. Physical mapping of genomes of phages ϕ29, ϕ15 and PZA using hybridization. The partial digests of DNAs of ϕ29 (1), ϕ15 (2) and PZA (3) by restriction endonucleases *Hpa*I and *Hin*dIII were separated by electrophoresis in 0.8% agarose gel (B) and transferred to DBM paper. The blot was hybridized first with the nick-translated left-terminal fragment (1188 bp fragment F of the ϕ29 DNA *Msp*I digest) (A). Then it was washed in formamide and hybridized again with the nick-translated right-terminal fragment (273 bp fragment L of ϕ29 DNA *Hin*dIII digest) (C). The radioactive bands correspond to the positions of restriction sites mapped from the left (A) and the right (C) ends of the phages' genomes. The numbers on the left represent fragment sizes of the length standard (S) (mixture of *Eco*RI and *Hin*dIII digests of ϕ29 DNA). The position of the whole-length molecule (19.5 kb) is also indicated.

read in the opposite orientation and this enables additional confirmation of restriction site location. There is no overlap of mapped regions in the method of Smith and Birnstiel in one experiment.

Our method enables fast mapping of several related genomes in parallel. In this case only two relatively short fragments should be isolated, which hybridize to all analysed DNAs.

The comparison of physical maps of phages ϕ29, ϕ15 and PZA (fig.3) suggests that all have arisen from a common ancestor. Out of altogether 31 positions for restriction sites of *Eco*RI, *Hpa*I and *Hin*dIII, 12 are common for all 3 phages and 20 are common for at least 2 of them. The observed differences can be explained by accumulation of point mutations and minor deletions [one such deletion near the right terminus of ϕ15 DNA would explain the small shift in the bands of *Hin*dIII digest (fig.2C)]. This conclusion has been further substantiated by comparison of our preliminary nucleotide sequence data.

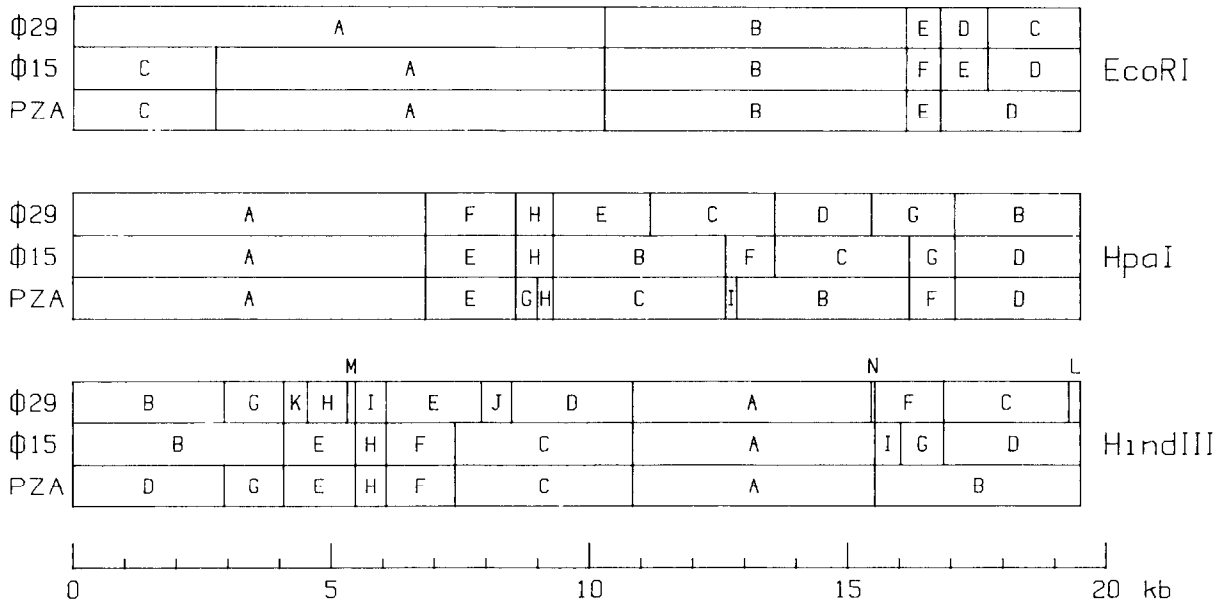

Fig.3. Comparison of physical maps of phages ϕ29, ϕ15 and PZA. Physical maps of ϕ29 have been published in [10], and *Eco*RI maps of ϕ15 and PZA are from [3]. *Hpa*I and *Hin*dIII maps of õ15 and PZA are derived from figs 1 and 2.

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