

A. Portolés R. López M. Espinosa (Editors)

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MODERN TRENDS IN BACTERIAL TRANSFORMATION AND TRANSFECTION

OPENING

Antonio Portolés

Dear friends,

It is my privilege to officially open this 3rd European Meeting on Bacterial Transformation and Transfection, and to welcome you to Granada.

The decision taken in Cracow, two years ago, for Spain to act as the host country, was indeed a great honor for us, and we can only hope that the high organizational levels found in the two previous meeting -in Portugal and in Poland, respectively- would be reproduced. This year has been a very difficult one for our country and, in many ways, this widened the range of our organizing problems. However, we have done our best, to arrange an efficient and friendly meeting. If there is something we can improve please tell us frankly.

I am deeply grateful to my friends and coworkers Rubens López and Manuel Espinosa, as well as to all the members of my group for their important cooperation.

Many thanks also, to the local organizing Committee and to the Granadian Authorities from the University, the Ayuntamiento and the Diputación for their kind receptions, and to the Patronato de la Alhambra for their invaluable help and generosity in allowing us to hold the Conference at this magnificent and rare place.

Little did Charles the Fifth think when he ordered the construction of this beautiful palace that it would one day host a Meeting like this. Do not forget there is a saying in Spanish: "Todo es posible en Granada" that is: "Everything is possible in Granada". I am sure you will appreciate these nice surroundings and I think they will immensely contribute to the success of our Meeting.

It is also my duty to point out that the Superior Council of Research, the Spanish Society for Microbiology, as well as the Ministries of Foreign Office and Information & Tourist Office, the EMBO and some industrial firms that have sponsored and supported the Meeting. Let me express to all of them my special gratitude for their economic cooperation.

And finally, our moment of the truth has arrived and the timetable must be followed; lectures, poster sessions, round tables and informal discussions will build up this 3rd European Transformation

Meeting.

I wish to thank you all for coming from so many different countries to participate and contribute in this Meeting. I am sure the exchange of scientific information will be very important, and we will make considerable inroads into the problems of the intimate mechanisms of the transfer of genetic material.

Meeting like this are always very helpful in increasing cooperation between scientists and establishing friendships that lead to the solution of many problems. Such a type of collaborative work and friendship is very important in a world like ours. As you say in English, two heads are better than one. Also this type of friendship is very important in ensuring that discoveries are applied for the benefit of mankind.

I sincerely hope everyone of you will feel at home here in Spain.

Thank you

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PHYSICO - CHEMICAL ASPECTS OF COMPETENCE

BIOLOGICAL AFFINITY OF PROTEASE-SENSITIVE
TRANSFECTING DNAs OF SMALL SIZE OF BACILLUS PHAGES

Hideo Hirokawa⁺, T. Shibata, J. Takagi⁺⁺ and T. Ando
Department of Microbiology, The Institute of Physical
and Chemical Research, Wako-shi, Japan

INTRODUCTION

Previously we reported that protease-sensitive transfecting DNAs were isolated from Bacillus phages, ϕ 29, ϕ 15, SP02, and ϕ 105(1). Transfective DNA of phage GA-1 has been reported also protease-sensitive(2). In addition to those phages, we have found that transfective DNAs of phage M2 and Nf, which were isolated and extensively studied in Japan, have similar characters to ϕ 29 DNA in transfection. Among these small phages, ϕ 29, ϕ 15, M2, and Nf, showed indistinguishable morphology and similar size of DNA molecules, 12×10^6 daltons. These evidences prompt us to study a biological affinity between such particular type of Bacillus phages.

The present communication will describe a) significant difference between DNA fragments formed by the action of restriction endonucleases and b) brief results of transfection of these DNAs in the presence of heterohelper phage.

MATERIALS AND METHODS

Phage ϕ 29 and ϕ 15 were originally given to us by B. Reilly and J. Ito, respectively. Phage M2 was isolated by one of us (J. T.) and Nf was found by N. Shimizu, *et al.* (5) independently. Temperature sensitive ϕ 29, used as helper, was kindly given by T. A. Trautner. Phage DNA was extracted by phenol in presence of sodium lauroyl sarcosinate according to previous paper(3). Transfection used was same to that of Spatz and Trautner(4). Restriction endonucleases, EcoRI and HindIII, were purchased of Miles, U.S. A. Reaction mixture of the enzyme treatment was described in the legend of plate I. Phage antisera for ϕ 29 and M2 were made by conventional method and given to us by T. Miura.

RESULTS

Biological Significances: Some properties of Bacillus phage DNAs are summarized in Table 1. It is obvious that DNA of small size phage, ϕ 29, ϕ 15, M2, and Nf, had similar characters except for the G-C contents. The G-C contents were derived by different analytical methods; thus the G-C differences may be simply a product the different analytical methods and not true biological difference. Although these phages were independently isolated in different places, they could be identical.

⁺ Present address: Office for Life Science Promotion, The Institute of Physical and Chemical Research, 2-28-8 Honkomagome, Bunkyo-ku, Tokyo and Department of Hygiene, Teikyo University School of Medicine, Itabashi, Tokyo, Japan.

⁺⁺ Department of Biology, International Christian University, Mitaka-shi, Japan.

TABLE 1
TRYPSIN SENSITIVITY AND SOME BIOLOGICAL PROPERTIES
OF TRANSFECTING DNAs OF BACILLUS PHAGES

Phage	Temperate(t) Virulent(v)	M.W. of DNA $\times 10^6$ daltons	GC%	Trypsin sensitivity	DNA dose response	Transfection on rec ⁻⁴	Shape of DNA
$\phi 29$	v	12	36 ⁺	S	1	yes	cir
$\phi 15$	v	12	nt	S	1	yes	nt
M2	v	12	38 ⁺⁺	S	1	yes	cir
Nf ^a	v	12	39 ⁺⁺	S	1	yes	cir
GA-1 ^b	v	13	36 ⁺	S	1	yes	cir
$\phi 105^c$	t	25	43	S	1	yes	cir
SP02 ^d	t	26	43	S	1	yes	cir
SPP1 ^d	v	25	43	r	2	no	lin
SP50 ^d	v	97	44	r	3	no	lin
SP01	v	100	43	r	3	no	lin

a): Shimizu, N. et al. (5). b): Arwert, F. and Venema, G.(2). c): Rutberg, L. et al. (6). d): Data were obtained from references reported in the review by Traefner, T. A. and Spatz, H. Ch. (7).

+: calculated from density of trypsin treated DNA. ++: chemical analysis.
S: sensitive, r: resistance. cir: can form circular DNA, lin: linear DNA only.
nt: not tested.

TABLE 2
PATTERNS OF PHAGE SUSCEPTIBILITY ON RESISTANT STRAINS
AND OF SENSITIVITY AGAINST PHAGE ANTISERUM

Phage resistant strain	No. of clone tested	Phage growth			
		$\phi 29$	$\phi 15$	M2	Nf
B. subtilis sus ⁺ 3/ $\phi 29$	6	-	-	-	-
" " / M2	6	-	-	-	-
B. amyloliquefaciens/ $\phi 29$	5	-	-	-	-
" " / $\phi 15$	6	-	-	-	-
B. subtilis tolA/ $\phi 15$	1	+	-	+	+
" " tol B/ $\phi 29$	1	-	-	-	-
Phage antiserum against $\phi 29$		S	R	R	R
" " against M2		R	R	S	S

Neutralization of phages by antiserum and growth of phages on phage resistant strain were shown in Table 2. Resistant strains to one of each of the phages resistant to all other three phages, except tol A/ ϕ 15 strain which permitted growth of the other phages. Neutralization of phages by antiserum clearly indicated that there were three phage groups. Nf was much more sensitive to M2 antiserum than that of M2.

DNA fragments formed by restriction endonucleases: The restriction enzyme patterns generated by EcoRI or HindIII were analyzed for each of those DNAs in order to determine their similarities. The gel on the left side of Plate 1 reveals ϕ 29, M2, and Nf gave clearly different DNA fragments with EcoRI treatment. The ϕ 29 DNA gave four bands, however, when we treated the DNA first with trypsin then with EcoRI we obtained a different pattern. One of the four bands disappears and two new bands are formed (small fragments are indicated by arrows). This suggested that the second band (from top) of the nontrypsinized DNA was composed two fragments which were bound together with a protein. M2 and Nf DNAs were further analyzed by EcoRI using different times of treatment, shown right side of the Plate 1. The main upper and low fragments of M2 and Nf behaved in a similar way, however, both fragments of Nf were slightly larger than those of M2. The middle two fragments of M2 can be seen in short treatment time, whereas the two middle fragments of Nf can be seen after pronase treatment. They were derived from the uppermost fragment that can be seen just above the position of the main upper fragment. It is obvious that the two middle fragments of Nf were bound together with a protein which was very stable under the present condition.

HindIII gave entirely different fragments (Plate 2). Here, the difference between M2 and Nf in fragments appeared to be clear.

Helper transfection: Transfective DNAs of ϕ 29, M2, and Nf, were treated by trypsin (300 μ g/ml) and were added to competent culture of Bacillus subtilis strain 222 with a helper (bp-1, a ts mutant of ϕ 29) at moi 5. Number of infective centers was shown in Table 3. All trypsinized DNAs gave no infective center. Homologous helper transfection, trypsinized ϕ 29 DNA and bp-1 phage, gave many infective centers. Although numbers of infective centers were few, heterologous helper transfection gave raise to significant number of infective centers in the case of M2 and Nf. These results suggested that M2 or Nf DNAs are functionally complemented by ϕ 29 temperature sensitive mutant, bp-1.

DISCUSSION

Since we have found an unique phenomenon, protease sensitive of transfecting DNA in small size of Bacillus phages, survey of a relation between those phages is becoming important feature in terms of phage evolution which is similar to the relation between ϕ x174 and S13(8). ϕ 29, M2, and Nf have about same size of DNA molecules, 12×10^6 daltons, which have the potential for about twenty genes.

TABLE 3
TRANSFECTION BY TRYPSINIZED DNAs WITH HELPER PHAGE

Trypsinized DNA	μg per plate	No. of infective centers per plate at 46C	
		with helper	without helper
$\phi 29$	0.5	>3000	0
M2	0.2	24	0
Nf	0.5	23	0
no DNA	0	0	0

Competent cells: *Bacillus subtilis* 222.

Helper phage: bp-1, $\phi 29$ temperature sensitive mutant, infected at moi 5.
Transfection: DNA, 0.1 ml and helper phage, 0.1 ml, was added to competent cells, 0.2 ml, at same time. Incubation time was 40 min at 30 C. After DNase-treatment, 50 $\mu\text{g}/\text{ml}$, then 0.2ml of aliquot was plated and incubated at 46 C.

In order to know the gene arrangements, we have started investigate the distribution of target site for restriction endonucleases in such similar phage DNAs. Analysis of DNA fragments produced by restriction endonucleases is extremely useful to investigate total gene arrangements in similar character of DNA. In fact, DNA fragments derived from EcoRI or HindIII treatments were clearly different in those DNAs suggesting that total gene arrangement in each DNA might be different. In the meantime, J. Ito, et al. (9) reported that $\phi 29$ and $\phi 15$ have similar distribution of the target site to EcoRI, except that $\phi 15$ has one additional target in a particular DNA fragment. Taking their results, it would be considered that there were two types of gene arrangements, $\phi 29$ - $\phi 15$ and M2-Nf types. In detail, the similarity between M2 and Nf still uncertain, because DNA fragments produced by HindIII appeared to be significantly different.

Plate 1 and 2. Treatment of restriction endonuclease.

Reaction mixture: 0.1 μg DNA, 50 mM Tris HCl (pH 7.5), 5 mM MgCl₂, 0.2 mM EDTA, 5 mM mercaptoethanol, 50mM NaCl, EcoRI or HindIII (10 units), in 30 μl .

Reaction: 37 C, for 50 min or different time indicated in Plate 1. Reaction was terminated by addition of 10 μl of 0.1 M EDTA, and then 40% sucrose BPB mixture.

Electrophoresis: 0.7% agarose gel slab (140 x 140 mm), in the presence of 0.5 μg per ml of ethidiumbromide. 110V, for 2 hr, at room temperature.

PLATE 1

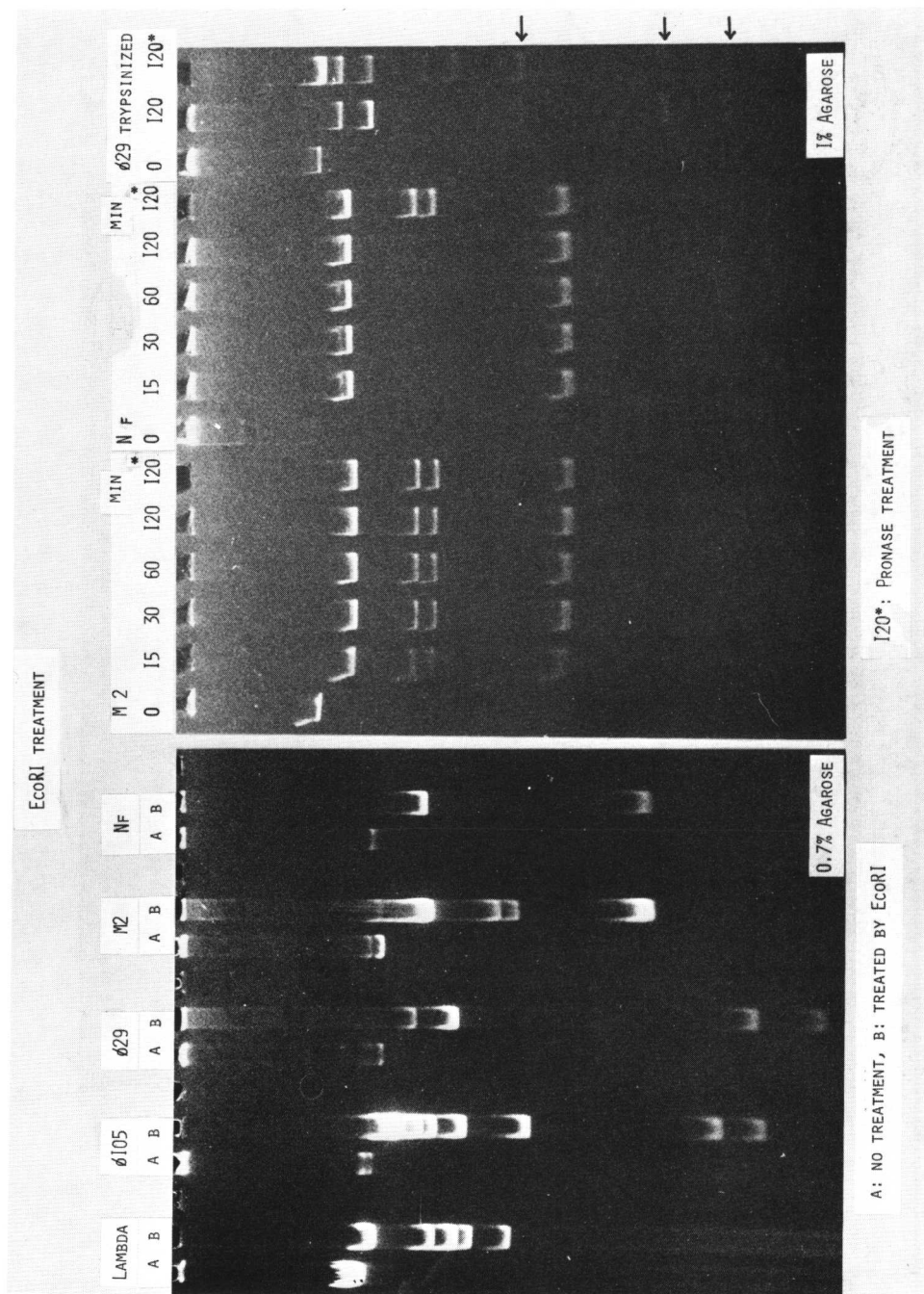
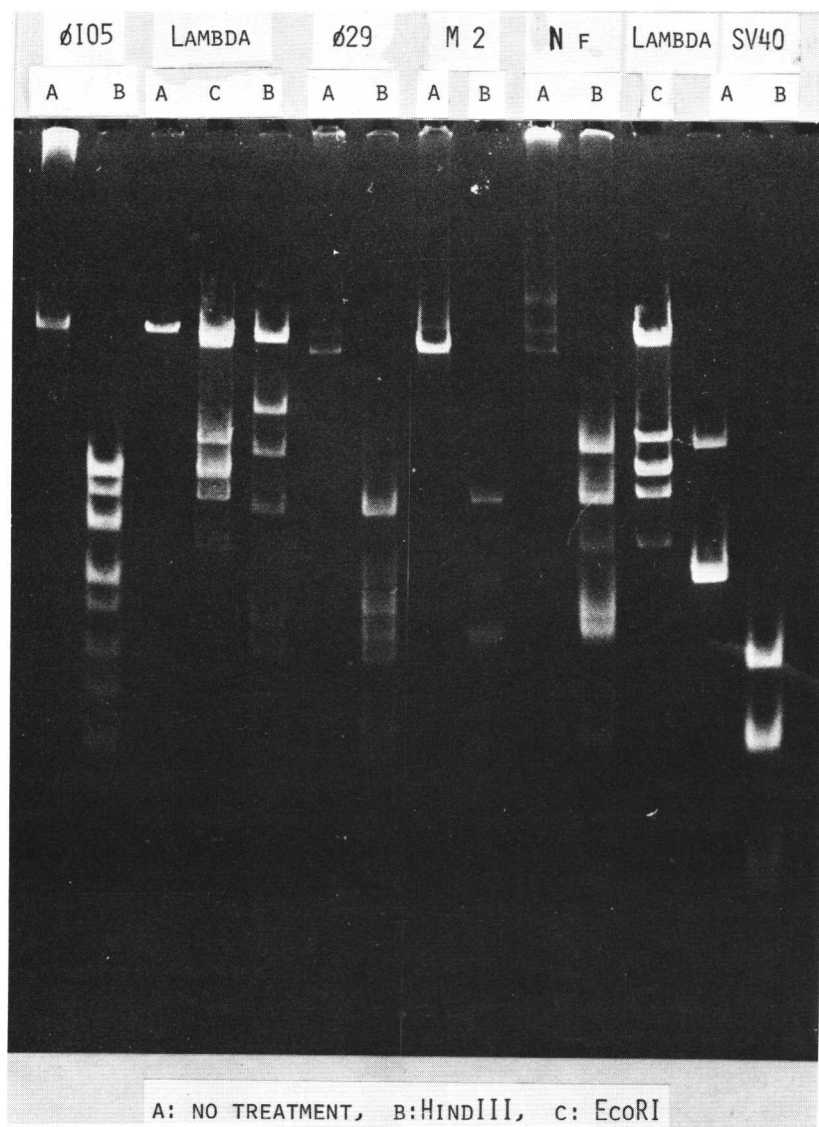


PLATE 2



It was obvious that $\phi 29$ was different from either M2 or Nf by means of immunologically and DNA fragments derived from restriction endonucleases. However, a biological affinity might exist between those phages which was indicated by heterohelper transfection.

As well as an ability of marker rescue in heterohelper transfection using different mutants, hybridization of DNA between those phages, and local melting pattern in whole DNA and in fragments derived from restriction endonucleases are under investigation.

SUMMARY

Small size of Bacillus phages, $\phi 29$, $\phi 15$, M2, and Nf were divided into three immunological groups. The DNA fragments produced by two restriction endonucleases, EcoRI and HindIII, revealed different distributions of enzyme target sites in each phage DNA. These data suggested a different gene arrangement for each of the DNAs. Regardless of arrangements, genetic complementation in heterohelper transfection occurred between M2 or Nf DNAs and a temperature sensitive mutant of $\phi 29$ phage.

REFERENCES

1. Hirokawa, H. (1974) Proc. II European Meeting of Transformation and transfection in Microorganisms, Krakow.
2. Arwert, F. and Venema, G. (1974) J. Virol. 13, 584.
3. Hirokawa, H. (1972) Proc. Natl. Acad. Sci. 69, 1555.
4. Spatz, H. Ch. and Trautner, T. A. (1970) Molec. gen. Genet. 109, 84.
5. Shimizu, N., Miura, K. and Aoki, A. (1970) J. Biochem. (Tokyo) 68, 277.
6. Rutberg, L. and Rutberg, B. (1970) J. Virol. 5, 604.
7. Trautner, T. A. and Spatz, H. Ch. (1973) Current Topics in Microbiology and Immunology, 62, 61.
8. Godson, G. N. (1973) J. Mol. Biol. 77, 467.
9. Ito, J., Kawamura, F. and Yanofsky, S. (1976) Virology, 70, 37.

