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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 Ministeries 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

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INTRODUCTION

Previously we reported that protease-sensitive transfecting DNAs were isolated from Bacillus phages, ϕ29, ϕ15, SPO2, and ϕ105(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 ϕ29 DNA in transfection. Among these small phages, ϕ29, ϕ15, M2, and Nf, showed indistinguishable morphology and similar size of DNA molecules, 12 x 10^6 daltons. These evidences prompt us to study a biological affinity between such particular type of Bacillus phages. The present comunication 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 ϕ29 and ϕ15 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 ϕ29, 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 antiserums for ϕ29 and M2 were made by conventional method and given to us by T. Miura.

RESULTS

Biological Sigificances: Some properties of Bacillus phage DNAs are summarized in Table 1. It is obvious that DNA of small size phage, ϕ29, ϕ15, M2, and Nf, had similar charactors 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.

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TABLE 1

TRYPSIN SENSITIVITY AND SOME BIOLOGICAL PROPERTES

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
ϕ29	v	12	36+	S	1	yes	cir
ϕ15	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
ϕ105[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 Trafner, T. A. and Spatz, H. Ch. (7).
+: calaulated 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 ϕ29	ϕ15	M2	Nf
B. subtilis sus^{+3}/ ϕ29	6	−	−	−	−
" " / M2	6	−	−	−	−
B. amyloliquefaciens/ ϕ29	5	−	−	−	−
" " / ϕ15	6	−	−	−	−
B. subtili tolA/ ϕ15	1	+	−	+	+
" " tol B/ ϕ29	1	−	−	−	−
Phage antiserum against ϕ29		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/ø15 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 ø29, M2, and Nf gave cearly different DNA fragments with EcoRI treatment. The ø29 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 disappers and two new bands are formed(small fragments are indicated by arrows). This suggested that the second band (from top) of the nontrypsininzed 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 upperest 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 entiraly different fragments (Plate 2). Here, the difference between M2 and Nf in fragments appeared to be clear.

Helper transfection: Transfective DNAs of ø29, M2, and Nf, were treated by trypsin (300μg/ml) and were added to competent culture of *Bacillus subtilis* strain 222 with a helper (bp-1, a ts mutant of ø29) at moi 5. Number of infective centers was shown in Table 3. All trypsinized DNAs gave no infective center. Homologous helper transfection, trypsinized ø29 DNA and bp-1 phage, gave many infective centers. Although numbers of infective centers were few, heterogous 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 ø29 temperature sensistive mutant, bp-1.

DISCUSSION

Since we have found an unique phenomenon, protease sensitive of transfecting DNA in small size of *Bacillus* phages, survay of a relation between those phages is becoming important feature in terms of phage evolution which is similar to the relation between øx174 and S13(8). ø29, 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
ϕ29	0.5	>3000	0
M2	0.2	24	0
Nf	0.5	23	0
no DNA	0	0	0

Competent cells: <u>Bacillus subtilis</u> 222.
Helper phage: bp-1, ϕ29 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 µg/ml, then 0.2ml of aliquot was plated and incubated at 46 C.

In order to know the gene arrengements, 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 extremly useful to investigate total gene arrengments in similar charactor of DNA. In fact, DNA fragments derived from EcoRI or HindIII treatments were clearly different in those DNAs suggesting that total gene arrengment in each DNA might be different. In the meantime, J. Ito, <u>et al.</u>(9) reported that ϕ29 and ϕ15 have similar distribution of the target site to EcoRI, except that ϕ15 has one additional target in a particular DNA fragment. Taking their results, it would be considered that there were two types of gene arrengments, ϕ29-ϕ15 and M2-Nf types. In detail, the similarity between M2 and Nf still unceatin, because DNA fragments produced by HindIII appeared to be significantly different.

Plate 1 and 2. Treatment of restriction endonuclease.
<u>Reaction mixture</u>:0.1 µg DNA, 50 mM Tris HCl (pH 7.5), 5 mM MgCl, 0.2 mM EDTA, 5 mM mercaptoethanol, 50mM NaCl, EcoRI or HindIII (10 units), in 30 µl.
<u>Reaction</u>: 37 C, for 50 min or differenet time indicated in Plate 1. Reaction was terminated by addition of 10 µl of 0.1 M EDTA, and then 40% sucrose BPB mixture.
<u>Electrophoresis</u>: 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

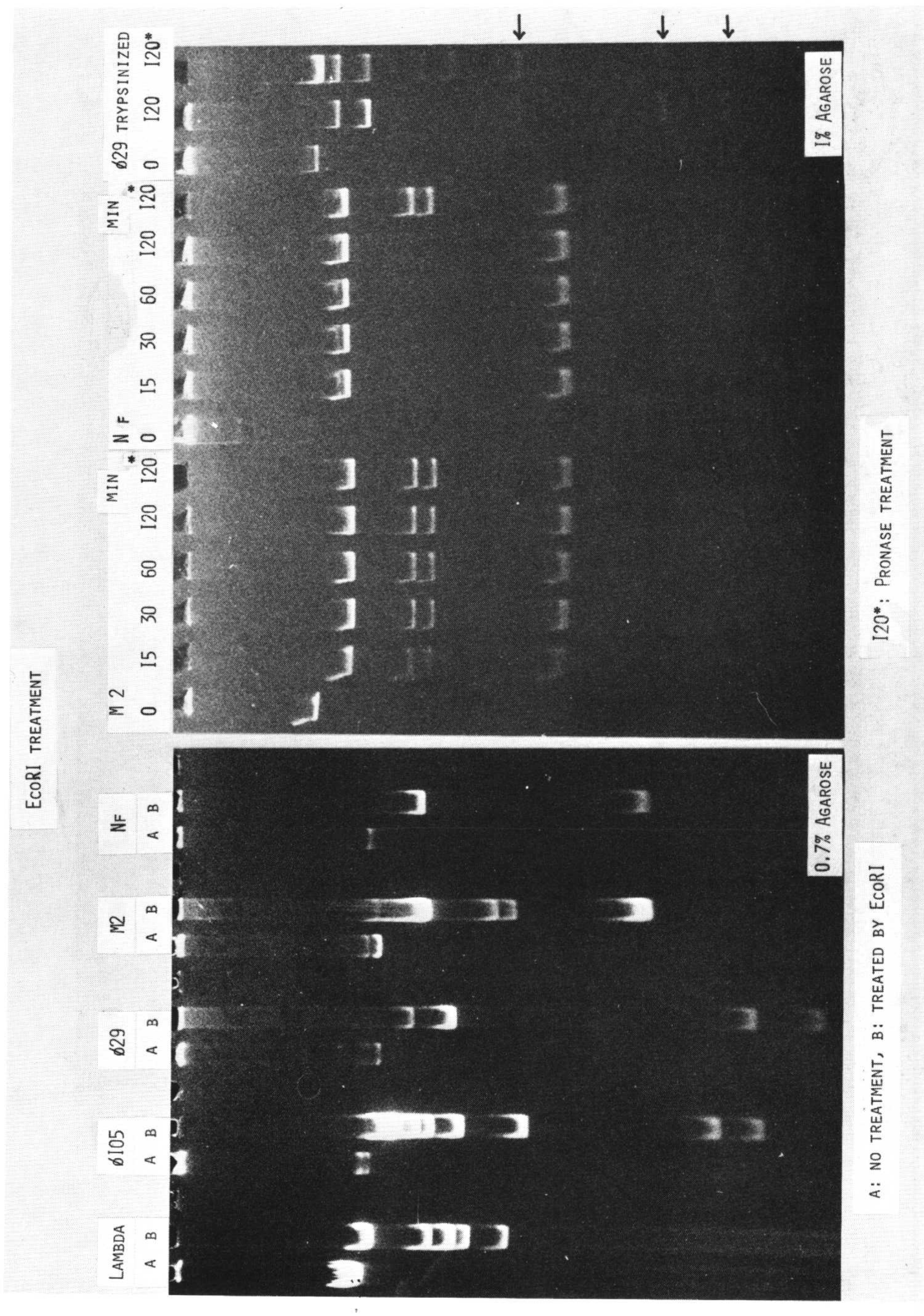
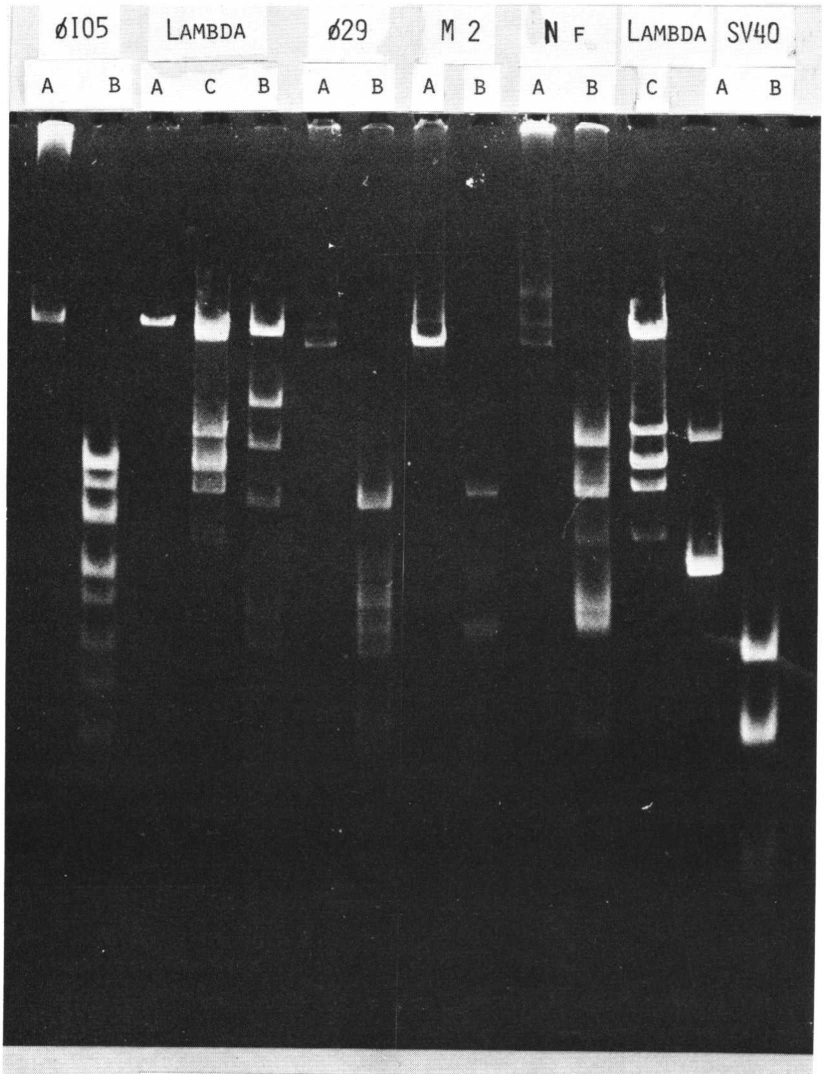

EcoRI TREATMENT
LAMBDA
A B
ø105
A B
ø29
A B
M2
A B
NF
A B
0.7% AGAROSE
A: NO TREATMENT, B: TREATED BY EcoRI
M 2
0 15 30 60 120 120*
MIN
NF
0 15 30 60 120 120*
MIN
ø29 TRYPSINIZED
0 120 120*
1% AGAROSE
120*: PRONASE TREATMENT

PLATE 2



A: NO TREATMENT, B:HINDIII, C: EcoRI

It was obvious that ϕ29 was different from either M2 or Nf by means of immno-logicaly 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, ϕ29, ϕ15, M2, and Nf were divided into three immunological groupes. The DNA fragments produced by two restriction endo-nucleases, 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 occured between M2 or Nf DNAs and a temperature sensitive mutant of ϕ29 phage.

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