FORMATION OF PLASMID F' RECOMBINANTS IN RECOMBINATION-DEFICIENT Escherichia coli CELLS AND THEIR PROPERTIES

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By conjugating cells carrying the F'his plasmid with cells containing the F'lac plasmid merodiploids with the F'His F'lac complex were obtained. The plasmid genes of these merodiploids are donated together to recipient cells and eliminated together from host cells both spontaneously and after treatment with acridine orange. Since the plasmid complex was formed in cells mutant for the rec A gene, it is suggested that recombination of plasmids can take place in the absence of the product of the rec A gene.

KEY WORDS: plasmid; conjugation; recombination; transconjugant; merodiploid; segregation.

Recombination between nucleotide sequences of DNA of different F' plasmids has been shown in several cases [1, 4, 7]. At the same time there is evidence that these plasmids do not possess their own recombination system and that their recombination in Escherichia coli depends on the product of the chromosomal rec A gene, for it is completely absent in cells mutant for this gene [5, 6]. According to other data, recombination of R plasmids also depends on the product of the rec A gene [3].

The formation of plasmid F' recombinants in E. coli cells mutant for the rec A gene was studied in this investigation.

EXPERIMENTAL METHOD

Plasmids F'lac and F'his used for hybridization were contained in cells of strains E. coli JC 5485 F'lac$^+$/ rec A lac his trp Str^s and JC 5441 F'his$^+$/rec A lac his trp Str^r respectively. To determine the conjugative properties of the hybrid F'lac F'his plasmid, the following strains of E. coli were used: AB 2463 F$^-$ thr leu pro A his arg E rec A, C 600 F$^-$ thr leu thi, J 62 F$^-$ pro try his, P 678 F$^-$ thr leu thi, PA 256 F$^-$ pro his arg F pur A nal^r, and PA 373 F$^-$ met A arg F thr leu his mal^r.

Hybridization of the plasmids was carried out by conjugating cells of 3-h cultures of strain JC 5485 F'lac used as donors with phenocopies of F$^-$ cells of strain JC 5441 F'his$^+$, grown in liquid minimal medium with aeration for 24 h at 37°C. Conjugation mixtures of donor cells and recipient cells (F$^-$ phenocopies) were prepared in the ratio of 1:5. The mixtures were incubated for 30 min at 37°C and then plated out on selected media, for selection of His$^+$ Lac$^+$ Str^r transconjugants. The sensitivity of cells containing the plasmids to phages f2, MS2, and 11 was tested by the method of agar layers. To determine the frequency of spontaneous segregation of cells which had lost the hybrid F'his F'lac plasmid, cells carrying this plasmid were grown in liquid minimal medium for 18 h at 37°C, then reseeded into nutrient broth, grown for 2.5 h at 37°C (for 5-7 generations), after which specimens of dilutions were seeded on nutrient agar or Endo's agar. After incubation of the seedings for 24-48 h cells of the growing colonies were analyzed for the presence or absence of His$^+$ Lac$^+$ markers by seeding on minimal media of appropriate composition. The hybrid plasmid was eliminated from the cells by acridine orange in doses of 50-150 μg/ml. The Rec$^-$ genotype of the cells was determined from their sensitivity to ultraviolet light.

EXPERIMENTAL RESULTS

As the results of conjugation of JC 5485 F'lac$^+$ cells with JC 5441 F'his$^+$ cells colonies of His$^+$ Lac$^+$

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TABLE 1. Frequencies of Transfer of His^+ and Lac^+ Markers by His^+ and Lac^+ Merodiploids in Conjugations with Recipient Cells of Different Strains of E. coli

Conjugations		Frequency of transconjugants (per donor cell) and their structure				
		His^+ Lac^+	His^+		Lac^+	
donors	recipients	frequency	frequency	% Lac^+	frequency	% His^+
JC5441his+	AB2463	—	1,0	0	—	—
	PA373	—	1,0	0	—	—
JC5485lac+	AB2463	—	—	—	0,9	0
	PA373	—	—	—	0,6	0
Merodiploid 2	AB2463	0,5	0,5	100	0,6	97
His+Lac+	PA256	0,5	0,3	99	0,6	98
	PA373	$0,4 \cdot 10^{-1}$	$0,2 \cdot 10^{-1}$	100	$0,6 \cdot 10^{-1}$	100
Merodiploid 3	AB2463	0,7	0,4	—	0,7	—
His+Lac+	PA256	0,4	0,3	—	0,4	—
	PA373	$0,2 \cdot 10^{-1}$	$0,2 \cdot 10^{-1}$	—	$0,3 \cdot 10^{-1}$	—
Merodiploid 12	AB2463	0,3	0,2	100	0,5	98
His+Lac+	PA256	0,2	$0,6 \cdot 10^{-1}$	99	0,2	100
	PA373	0,1	$0,8 \cdot 10^{-1}$	100	0,1	100
Merodiploid 17	AB2463	0,1	0,1	100	0,1	99
His+Lac+	PA373	0,7	0,8	99	0,8	100
Merodiploid 27	AB2463	0,5	0,25	100	0,4	98
His+Lac+	PA373	0,8	1,0	100	0,9	100

TABLE 2. Frequency of Chromosome and Plasmid Transfer by PA 256 His^+ Lac^+ Merodiploids

Conjugations		Frequency of recombinants and meridiploids (per donor cell)				
donors carrying plasmid markers	recipients	Leu^+	Met^+	Thr^+	Trp^+	His^+ Lac^+
Merodiploid 3a	PA373	$0,3 \cdot 10^{-1}$	$0,5 \cdot 10^{-1}$	$0,4 \cdot 10^{-3}$	—	0,4
(PA256 his+lac+)	J62	—	—	—	$1,1 \cdot 10^{-3}$	1,0
Merodiploid 12a	PA373	$0,3 \cdot 10^{-1}$	$0,3 \cdot 10^{-1}$	$0,2 \cdot 10^{-1}$	—	0,2
(PA256 his+lac+)	J62	—	—	—	$1,5 \cdot 10^{-3}$	0,6
	C600	$3,3 \cdot 10^{-3}$	—	$2,5 \cdot 10^{-3}$	—	—
	P678	$2,4 \cdot 10^{-3}$	—	$3,2 \cdot 10^{-3}$	—	—

Str^r transconjugants were grown on selective media with a frequency of 6.5×10^{-4}–1.5×10^{-5} per donor cell. After purification of the transconjugants by seeding on analogous medium, they were tested for the presence of plasmid and chromosome markers and also for sensitivity to phages f 2, MS 2, and 11. The tested His^+ Lac^+ Str^r transconjugants had the genotype of cells of the original JC 5441 cultures, they had markers of both plasmids, and they were sensitive to phages f 2 and MS 2 but resistant to phage 11. The preliminary conclusion was drawn from these data that the isolated transconjugants are His^+ Lac^+ merodiploids containing both the plasmids – F'his and F'lac.

To obtain data on the nature of coupling of the F'his and F'lac plasmids in the cells of the segregated His^+ Lac^+ Str^r merodiploids, the latter were tested 20 at a time for their ability to donate His^+ Lac^+ markers simultaneously in subsequent conjugations with recipient cells AB 2463 F^-, PA 256 F^-, and PA 373 F^-. These experiments showed that five of the transconjugants (Nos. 2, 3, 12, 17, and 27) had the ability to transfer His^+ and Lac^+ markers simultaneously to recipient cells of different strains. The data on the frequencies of transfer and the character of transfer in these conjugations are given in Table 1. As this table shows, the cells of all merodiploids carried out simultaneous transfer of His^+ and Lac^+ markers with fairly high frequency. Simultaneous transfer of the two markers in these conjugations also was established by separate selection of the merodiploids relative to one of the plasmid markers (His^+ or Lac^+). The frequency of appearance of these merodiploids was just as high as that of merodiploids selected for both plasmid genes. Analysis of their structure showed that the His^+ merodiploids in 100% of cases were simultaneously Lac^+ merodiploids, and that the Lac^+ merodiploids, in turn, were His^+ merodiploids in 98–100% of cases. Furthermore, investigation of the phage sensitivity of merodiploids of each of these classes showed that they are all sensitive to phages f 2 and MS 2 and resistant to 11. Consequently, these results were evidence that merodiploid cells Nos. 2, 3, 12, 17, and 27 contain both F'his and F'lac plasmids, and in a linked form. However, it could not be decided from these findings whether the linked plasmid is in an autonomous state in cells conventionally defined as merodiploids. Preliminary data on the state of this plasmid could be obtained by comparing the frequency of plasmid and chromosomal transfer. Since merodiploids Nos. 2, 3, 12, 17, and 27, possessing the linked plasmid complex F'his F'lac are mutants for the rec A gene, which rules out any possibility of explaining the ability of the plasmids to mobilize chromosomes for transfer, in the next experiments the F'hisF'lac plasmid complex of each mero-

diploid was transferred by conjugation crosses to cells of strain PA 256 F^-, characterized by normal recombining ability. The PA 256 donors containing the F'hisF'lac plasmid complex thus obtained were then tested for plasmid and chromosome transfer in crosses with PA 373 F^-, J 62 F^-, C 600 F^-, and P 678 F^- cells, with selection of the His^+ Lac^+ transconjugants and the Thr^+, Leu^+, Met^+, and Trp^+ recombinants. The results of these experiments are given in Table 2.

As Table 2 shows, the frequency of plasmid transfer in these crosses was considerably higher than the frequency of chromosome transfer. Moreover, some of the transconjugants and recombinants of all classes were spot checked for their behavior toward phages f2 and MS2. All were found to be sensitive to these phages, a clear indication of the autonomous existence of the His^+ Lac^+ plasmid complex.

In the final experiments to prove the autonomous and linked state of the F'hisF'lac plasmid complex, spontaneous segregation by merodiploids Nos. 2, 3, 12, 17, and 27 of cells deficient in this complex and the frequency of this segragation after treatment of His^+ Lac^+ transconjugants with acridine orange were determined.

Merodiploids Nos. 2, 3, 12, 17, and 27 spontaneously segregated both His^- and Lac^- cells. The frequency of spontaneous segregation of Lac^- cells by His^+ Lac^+ cultures was 0.3-1.1%; analysis of the genetic structure of these segregants showed that loss of the Lac^+ marker also was accompanied by loss of the His^+ marker, loss of donor ability, and of sensitivity to phage f 2. The Lac^+ His^- segregants became sensitive to recipient phage 11, and in crosses with HfrH and JC 5441 F'his^+/Str^S donor cells they behaved as recipient cells. Introduction of the plasmid complex from PA 256 cells into spontaneous Lac^- His^- segregants completely restored their His^+ Lac^+ phenotype, their sensitivity to phage f 2, and their ability to transfer the plasmid complex to recipient cells in subsequent conjugations.

Treatment of the His^+ Lac^+ merodiploids with acridine orange led to an increase in the frequency of segregation of Lac^- cells to 25-77%. Testing of 100 clonal cultures arising from cells remaining after acridine orange treatment of Lac^+ showed that they all preserved the His^+ Lac^+ phenotypes, were sensitive to phage f 2, and donated both markers with high frequency in crosses with recipients. The clonal cultures arising from cells remaining after acridine orange treatment of Lac^- were heterogeneous in their genetic structure; Two thirds of the Lac^- clones were simultaneously His^-, resistant to phage f 2, and had no donor ability; they were F^- cells, for in crosses with donor cells they formed genetic recombinants, although with low frequency. In their properties they were similar to spontaneous His^- Lac^- segregants. The remainder (one third) of these Lac^- clones retained their His^+ phenotype and sensitivity to phage f 2, and in crosses with Hfr or F^- cells they exhibited neither recipient nor donor ability. Possibly in the segregant cells of this class only the Lac^+ marker was lost with the tra plasmid segments.

It can be concluded from a generalization of the results of these experiments that the constructed F'his F'lac plasmid complex is a stable recombinant structure. Since the formation of this complex takes place in rec A cells in which the product of the rec A gene is absent, it can tentatively be suggested that F' plasmids possess their own recombination system or IS nucleotide sequence, which is responsible for the "illegitimate" recombination of these plasmids [2].

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