# ESCHERICHIA COLI GENE TRANSFER TO UNRELATED BACTERIA BY A HISTIDINE OPERON - RP1 DRUG RESISTANCE PLASMID COMPLEX

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SUMMARY. Genetic and physical evidence indicates that the Pseudomonas aeruginosa drug resistance factor, RP1, may promote the transfer of Escherichia coli histidine genes. The RP1 mobilized histidine genes are functionally expressed in diverse Salmonella typhimurium and P. aeruginosa histidine auxotrophs. The histidine operon - RP1 complex may be transferred back to E. coli strains from Pseudomonas.

The E. coli sex factor, F, has been shown to form F-prime (F') factors containing segments of the E. coli chromosome which are cotransferred with F to other related bacteria. However, as with F, the host range of these F' factors is limited to the Enterobacteriaceae. The construction and behavior of these F' factors have recently been the subject of a comprehensive review (1). The broad host range demonstrated for the Pseudomonas drug resistance factor, RP1, (2,3) suggested to us the interesting possibility of its use for the transport among diverse bacteria of genes unrelated to those specifying drug resistance. We report here an instance of gene pickup involving the apparent addition of the E. coli histidine operon gene cluster to the RP1 drug resistance plasmid. This complex, designated RP1I-42, will functionally complement mutations in the histidine biosynthetic pathway in E. coli, S. typhimurium LT2 and P. aeruginosa strains.

MATERIALS AND METHODS. A multiply auxotrophic E. coli strain, JC1553(KLF3-1), which is recombination deficient (recA) was received from Barry Wanner, University of Michigan. A derivative strain, designated JC1553, which had spontaneously lost the KLF3-1 histidine plasmid, was selected on the basis of its resistance to male specific phage f2. JC1553 is a histidine, leucine, methionine and arginine requiring auxotroph and will not act as a donor in

conjugal mating experiments with E. coli AB1132, an $F^-$ recipient. AB1132 is a threonine, leucine, proline, histidine and methionine requiring strain used in our laboratory. S. typhimurium LT2 strains having characterized mutations in the Salmonella histidine operon were received from Harvey Whitfield, University of Michigan and John Loper, University of Cincinnati. P. aeruginosa strains having mutations in the Pseudomonas transductional linkage groups described by Mee and Lee (4) were kindly provided by John Loper. Other bacteria used have been described in previous reports from our laboratory (3,5). Growth of donor cells for mating experiments was done by inoculating minimal solid medium supplemented with the antibiotic, carbenicillin, and required amino acids except histidine. After overnight growth at 37°C, a turbid suspension was prepared from this culture and inoculated into TGE broth medium. The broth culture contained approximately $1 \times 10^8$ cells per ml when used for mating. The composition of TGE, Vogel and Bonner minimal medium and mating conditions were as described previously for the RP1 plasmid (3). In our laboratory RP1 was formerly designated R1822.

RESULTS. Gene pickup of the E. coli histidine operon is presumed to have occurred in an E. coli HfrH(RP1) strain. This strain was constructed by mating P. fluorescens PF015.4(RP1) with E. coli HfrH as indicated in Table 1, mating no. 1. Selection against the psychrophilic donor of RP1 was at 37°C on carbenicillin containing medium. E. coli HfrH(RP1), an exconjugant of this mating which was f2 phage sensitive and expressed the RP1 plasmid determinants specifying carbenicillin, tetracycline, neomycin/kanamycin resistances as well as RP1 dependent PRR1 RNA phage (5) and PRD1 DNA phage sensitivities (6), was next mated as indicated in Table 1, mating no. 2. Exconjugants from this mating were selected on streptomycin containing minimal medium supplemented with amino acids required by JC1553 except histidine. JC1553 is streptomycin resistant (200 μg/ml). The histidine independent exconjugants were purified by several serial clonal isolations

Table 1. RP1 Plasmid Matings.[1,2]

| Mating No. | Donor strain x recipient strain | | Selection[3] | Exconjugants /donor | Indirect testing |
|---|---|---|---|---|---|
| 1 | *P. fluorescens* PF015.4(RP1) x *E. coli* HfrH | | CbR | $2 \times 10^{-4}$ | |
| 2 | *E. coli* HfrH(RP1) x *E. coli* JC1553 | | his | $1 \times 10^{-7}$ | |
| 3 | *E. coli* JC1553(RP1I-42) | | his | $1 \times 10^{-2}$ | 20/20 CbR |
| | x *E. coli* AB1132 | | CbR | $1 \times 10^{-2}$ | 20/20 his |
| 4 | *E. coli* AB1132(RP1I-42) x *E. coli* JC1553 | | his | $1 \times 10^{-2}$ | 20/20 CbR |
| 5 | *E. coli* JC1553(RP1I-42) | | his | $1 \times 10^{-4}$ | |
| | x *S. typhimurium* | OG1302 | " | " | |
| | | D1242 | " | " | |
| | | DC129 | " | " | 20/20 CbR |
| | | C527 | " | " | |
| | | B243 | " | " | |
| | | HA134 | " | " | |
| | | A816 | " | " | |
| | | F3020 | " | " | |
| | | IE640 | " | " | |
| | | C527 | CbR | $1 \times 10^{-3}$ | 2/20 his |
| 6 | *E. coli* JC1553(RP1I-42) | | his | $2 \times 10^{-8}$ | 10/10 CbR |
| | x *P. aeruginosa* PA067 | | CbR | $1 \times 10^{-4}$ | 0/20 his |
| 7 | *P. aeruginosa* PA067(RP1I-42) | | his | $5 \times 10^{-4}$ | |
| | x *P. aeruginosa* | PL101 (I) | " | " | |
| | | GMA060 (IIA) | " | " | |
| | | GMA044 (IIB) | " | " | |
| | | GMA065 (III) | " | " | 20/20 CbR |
| | | GMA052 (IV) | " | " | |
| | | GMA037 (V) | " | " | |
| | | GMA065 | CbR | $1 \times 10^{-3}$ | 10/20 his |
| 8 | *P. aeruginosa* PA067(PR1I-42) | | his | $3 \times 10^{-8}$ | 10/10 CbR |
| | x *E. coli* AB1132 | | CbR | $5 \times 10^{-4}$ | 0/20 his |

[1] his = histidine independence

[2] CbR = resistance to carbenicillin, 500 ug/ml

[3] Selection of exconjugants occurred on minimal medium which included appropriate required amino acids. Histidine was omitted or included in supplemented minimal medium as specified in the text.

on the medium used for mating and finally tested for their nutritional requirements and RP1 determinants. Of 100 exconjugants purified and tested, several isolates were found which were histidine independent but still

required leucine, arginine and methionine. Of these, one exconjugant was f2 phage insensitive although maintaining the RP1 plasmid specified determinants. This isolate was designated JC1553(RP1I-42).

JC1553(RP1I-42) was mated with another histidine requiring E. coli strain as shown in Table 1, mating no. 3. For this, selection against the donor was done by omitting arginine from the medium. When exconjugants were selected for their carbenicillin resistance on medium containing histidine, all were found to be histidine independent as well. Similarly, when selection for histidine independence was done, all exconjugants tested were carbenicillin resistant. Thus, RP1 specified carbenicillin resistance and histidine independence were cotransferred. The frequency of exconjugant formation in this instance also exceeds that reported previously for intraspecific transfer of RP1 among E. coli (3) or that attainable when JC1553(RP1) is mated with AB1132 with selection for the aquisition of carbenicillin resistance by AB1132 (unpublished data). An AB1132(RP1I-42) exconjugant was purified and mated with JC1553 as shown for mating no. 4. Exconjugants were selected on minimal medium lacking the donor proline requirement at approximately the same frequency for the reverse JC1553(RP1I-42) x AB1132 mating. The JC1553(RP1I-42) exconjugants were tested and found to express all the RP1 determinants, in addition to histidine independence.

We next determined if E. coli JC1553(RP1I-42) would transfer RP1I-42 to S. typhimurium strains having mutations known to be located throughout the Salmonella histidine operon. The locations of these mutations shown in Table 1, mating no. 5, traverse the entire histidine operon from the operator proximal OG1302 to the distal IE640 loci. The frequency of histidine independent exconjugant formation was approximately the same for all recipients. When C527 exconjugants selected for histidine independence were subsequently tested for RP1 specified carbenicillin resistance, all tested were resistant. However, when C527 exconjugants were selected for the aquisition of carbenicillin resistance on histidine supplemented minimal medium, only

two of twenty tested were also histidine independent. Thus, unlike the intraspecific E. coli matings, cotransfer of the RP1 determinant and histidine independence was not complete.

We next mated E. coli JC1553(RP1I-42) with P. aeruginosa PA067 and the results of this pairing are shown in Table 1, mating no. 6. PA067 is a mutant having an uncharacterized histidine requirement. All exconjugants selected for histidine independence were also carbenicillin resistant. However, when selection was for transfer of carbenicillin resistance, none showed cotransfer of histidine genes. This observation considered with the variance in the frequency of exconjugant formation depending upon primary selection for histidine independence or carbenicillin resistance may indicate poor survival of histidine genes in the merozygote formed immediately after transfer. This result will be discussed later.

We next tested the ability of a P. aeruginosa PA067(RP1I-42) isolate to transfer histidine independence to other histidine requiring P. aeruginosa strains. The results of this are shown in Table 1, mating no. 7. For this we used as recipient bacteria streptomycin resistant representatives of each of the P. aeruginosa transductional linkage groups for histidine biosynthesis described by Mee and Lee (4). The strain nomenclature and corresponding transductional linkage group indicated in parentheses is shown in Table 1. As shown in the table, the frequency of exconjugant formation was approximately the same for all the histidine mutants when selection was for histidine independence. Furthermore, when histidine independent GMA065 exconjugants were tested further, all were carbenicillin resistant. When primary selection was for carbenicillin resistance on histidine supplemented minimal medium, half of the isolates tested were subsequently found to be histidine independent. Thus, as observed previously with Salmonella recipients, cotransfer of histidine independence with the RP1 determinant was less than unity when primary selection was for carbenicillin resistance.

The variance in the frequency of histidine and carbenicillin transfer

(fewer histidine independent exconjugants than carbenicillin resistant) when carbenicillin selection was used raised the question of whether the E. coli histidine genes were integrated into the RP1 plasmid. An alternate mechanism is possible whereby a separate plasmid containing histidine genes is mobilized by the RP1 plasmid. The distinction here respectively would be between the behavior of a cointegrate or aggregate plasmid as described by Clowes (7). To provide information concerning these possibilities we labeled JC1553, JC1553(RP1) and JC1553(RP1I-42) with methyl-$^{3}$H-thymine and extracted the radioactive DNA using the procedure of Guerry, LeBlanc and Falkow (8). This procedure is reported to yield primarily plasmid DNA and we included a JC1553 lysate here for confirmation. The 5 to 20% sucrose gradient profiles using these lysates are shown in Figure 1. The absence of significant peaks in the plasmid region for JC1553 confirm the observation

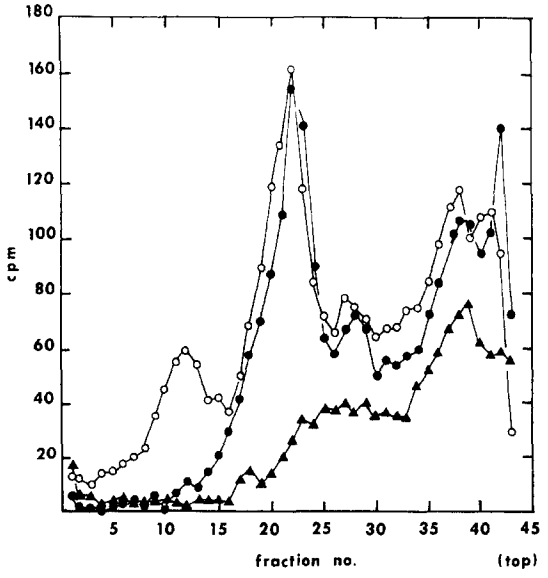


Figure 1. Sedimentation of lysates in a 5 to 20% sucrose gradient. JC1553, ▲——▲; JC1553(RP1), ●——●; JC1553(RP1I-42), ○——○. Gradients were produced by using a Beckman gradient forming device and contained in addition to sucrose, 0.02 M Tris, pH 7.4; 0.005 M EDTA; and 0.5 M NaCl. A 0.2 ml sample was added to the top and gradients were centrifuged at 39,000 rpm at 20°C for 65 min in a Spinco SW50.1 rotor. Samples were collected dropwise from the bottom onto filter paper squares and processed for acid precipitable cpm.

of Guerry et. al. (8). The JC1553(RP1) lysate yields peaks expected for covalently closed circular (CCC) and open circular (OC) forms of the plasmid. Furthermore, the distance separating the CCC and OC forms corresponds to that expected for duplex DNA of 39 megadaltons molecular weight using the ratios of sedimentation coefficients of configurational forms of duplex DNA reported by Clowes (7). When the JC1553(RP1I-42) lysate was examined, a peak not observed for RP1 or $R^-$ lysates was seen. The position of this peak seen in fraction 12 corresponds to that expected for an OC dimer of RP1. Thus, RP1I-42 is distinguished from RP1 by its tendency to form dimers as well as monomeric forms. However, the corresponding locations of both RP1I-42 and RP1 CCC DNA at fraction 22 show molecular weight differences occurring between these plasmids are below the resolution of these sucrose gradients (estimated at 2 megadaltons per fraction in the region of the monomer CCC peak). It also suggests the possibility that a part of the RP1 plasmid not associated with known functions has been replaced by the E. coli histidine genes. The sedimentation characterisitics of RP1 correspond to those reported previously by Grinsted et al. (2). Similarly, when P. aeruginosa $R^-$, RP1, or RP1I-42 lysates were compared as above, the fraction 12 fast sedimenting peak characteristic of RP1I-42 was observed but absent for the Pseudomonas RP1 lysate. However, these lysates also contained another plasmid as did $R^-$ PA067. This plasmid may be identical to that described previously by Pemberton and Clark for the related strain, P. aeruginosa PA02 (9). The physical characterization and comparison of RP1 and RP1I-42 will be the subject of another report (manuscript in preparation). The present studies with E. coli lysates, lead one to the tentative view that the RP1 - histidine operon complex is most likely a cointegrate plasmid with a tendency to form dimers during replication.

DISCUSSION. We have demonstrated the expression of E. coli genes concerned with histidine biosynthesis in an unrelated host, P. aeruginosa. The genetic transfer of this information is mediated by the RP1 plasmid and

apparently occurs as a result of RP1 forming a cointegrate plasmid with the E. coli histidine operon. This plasmid may be distinguished from RP1 on the basis of the presence of open circular dimers in lysates prepared by the sodium lauryl sulfate - salt precipitation method (8). The complete cotransfer of both histidine and RP1 genes observed for intraspecific E. coli matings supports this view. However, for interspecific matings between E. coli and Salmonella, or between E. coli and Pseudomonas, apparently less transfer occurs of histidine genes than RP1 determinants. This observation may merely reflect the preferential restriction of E. coli histidine genes in these recipient bacteria leaving intact the remaining unrestricted RP1 genes. Further evidence of this possibility is seen for the intraspecific Pseudomonas crosses wherein less apparent transfer of histidine genes than RP1 determinants is observed. In this instance, the E. coli nucleotide sequences may not be sufficiently compatible with Pseudomonas modification systems (10) to prevent preferential destruction by the exconjugant cells prior to replication and recircularization of the histidine genes transported by the RP1I-42 plasmid. With regard to the latter, we have not observed spontaneous loss of the histidine genes without RP1 curing following prolonged culture of either E. coli or Pseudomonas bacteria in dilute culture ($<1 \times 10^4$ cells/ml). Thus, RP1I-42, once established, persists during growth of the culture.

Our preliminary observations and those reported by Dunican and Tierney (11) may represent a significant mechanism facilitating microbial evolution. The intergenus transfer reported here for functions usually associated with chromosomal genes raises the interesting possibility that evolution of species may occur under certain selective conditions by virtue of the potential of broad host range plasmids for the transfer of large regions of chromosomal information. This potential has been demonstrated by both the RP1 mediated intergenus transfer of histidine genes and R1-19drd mediated intergenus transfer of nitrogenase activity (11).

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