COMPLEMENTATION OF NONCHEMOTACTIC MUTANTS OF *ESCHERICHIA COLI*[1]

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THE capacity to respond to stimuli by movement is a common property of living things. A simple form of response is a taxis, in which the organisms avoid, or accumulate near, the source of stimulation. Chemotaxis, the response to a chemical, has been observed in a wide variety of microorganisms, plants and animals (JENNINGS 1906; FRAENKEL and GUNN 1961), and has been recognized in the bacteria since the work of PFEFFER, ENGELMANN and other microbiologists in the latter part of the 19th century (for a review see WEIBULL 1960).

As an approach to elucidating the biochemical mechanisms of chemotaxis in *Escherichia coli*, we have isolated and characterized mutants which are defective in chemotaxis but fully motile (ARMSTRONG, ADLER and DAHL 1967). They were isolated by repeatedly picking the center of a swarm on a semisolid tryptone plate. Swarming on a semisolid medium results from chemotaxis toward the nutrients in the medium (ADLER 1966). These mutants do not show chemotaxis toward any substance tried.

In this paper we will show that the mutants may be divided into three complementation groups distinct from those, previously studied in *E. coli* (ARMSTRONG and ADLER 1967), which affect the structure and function of the flagella.

MATERIALS AND METHODS

The nonchemotactic mutants were isolated from the *E. coli* K12 strains AW330 and AW405 (ARMSTRONG *et al.* 1967). Nonmotile double mutants, either nonchemotactic-nonflagellated (*che⁻ fla⁻*) or nonchemotactic-paralyzed (*che⁻ mot⁻*), were isolated from the nonchemotactic mutants by treating with the phage chi, which attacks only motile bacteria, and examining the nonmotile survivors. The method is the same as that used by ARMSTRONG and ADLER (1967) to isolate nonmotile mutants. Isolates were tested for the proximity of the two sites by transduction with P1 grown on wild-type bacteria. Wild-type swarms will be obtained only if the two sites are cotransducible. Only those mutants yielding wild-type swarms were kept. These were tested for retention of the original mutation by transduction with P1 grown on their nonchemotactic parent. The mutants were also tested for flagella, both by staining and by agglutination with anti-flagella serum, for motility under the microscope, for sensitivity to chi phage, and for swarming on semisolid tryptone plates. The details of these tests have been described by ARMSTRONG *et al.* (1967).

The procedure for transduction with phage P1 has been described previously (ARMSTRONG and ADLER 1967).

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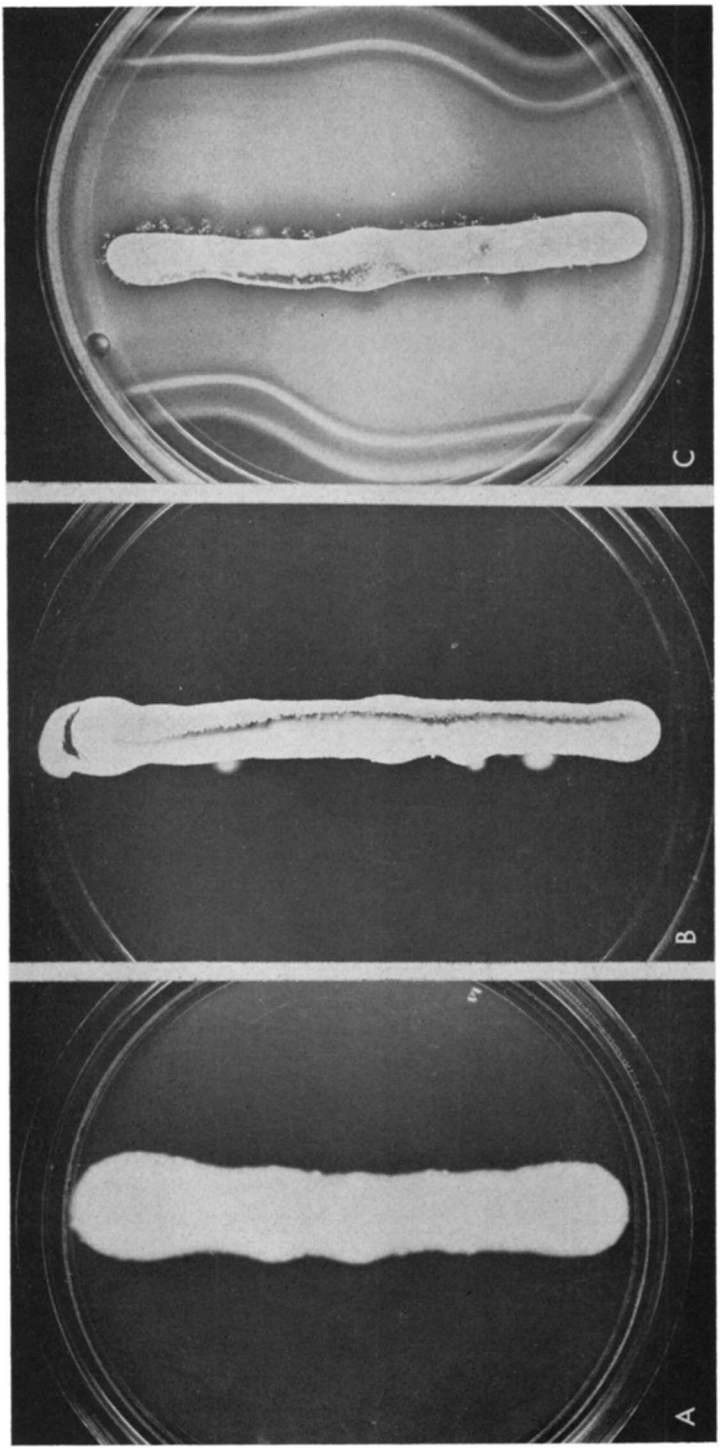

FIGURE 1.—A. Nonchemotactic mutant *cheA593*. B. Nonchemotactic-paralyzed double mutant

RESULTS

In genetic studies of motility in both Salmonella species and in *Escherichia coli* the method chosen for complementation has been abortive transduction (IINO 1958; ARMSTRONG and ADLER 1967). In an abortive transduction the donor mutant gene is introduced into a non-motile recipient by the phage, but fails to be incorporated or to multiply. If complementation occurs, the bacterium carrying the fragment will be motile, but as it swims out from the site of inoculation it leaves a trail of microcolonies containing its nonmotile descendants. If the donor is defective in the same gene as the recipient, trails usually do not form and complementation is said not to occur.

When a nonchemotactic mutant, which is normally motile and tends to spread on a semisolid agar plate just by random swimming (Figure 1A), is treated with P1 grown on the wild type, no trails are observed. The reason is that the nonchemotactic cells in both the inoculum and the trails are motile, and will all spread together to give a result which cannot easily be distinguished from an inoculum of nonchemotactic cells which have not been transduced.

To circumvent this problem we have constructed mutants with a second defect, leading to a loss of motility, sufficiently close to the initially mutated site that the two can easily be cotransduced by P1 (see details under MATERIALS AND METHODS). The double mutants are either nonchemotactic-nonflagellated (*che*$^-$ *fla*$^-$) or nonchemotactic-paralyzed (*che*$^-$*mot*$^-$). They do not spread from the site of inoculation (Figure 1B) and do give trails when transduced with P1 from wild-type bacteria (Figure 1C). This makes complementation studies feasible, as the double mutants can now be transduced with P1 from each of the nonchemotactic mutants, and should yield trails only when the donor belongs to a different complementation group.

When the double mutants were transduced with P1 grown on their nonchemotactic parents, trails did not form. This result shows that chemotaxis is necessary for the formation of trails. The bacterium made motile by the donor fragment must be attracted by the more favorable conditions away from the inoculum. This is in line with the observation of LEDERBERG (1956) that when abortive transductants are placed individually on a semisolid medium so that they are not in a crowded environment, the resulting microcolonies form clusters rather than trails.

Complementation tests between nonchemotactic mutants: Complementation tests on seven double mutants were carried out using P1 grown on 38 nonchemotactic donors. A positive response always consisted of 50 to 200 trails per plate (except in the case of *cheC497*, *fla*$^-$, see below). In a negative response no trails (or occasionally up to 3 or 4) were observed, though a slightly beaded edge to the

cheA593, *mot*$^-$. In both cases 0.1 ml of an overnight culture was streaked across a semisolid tryptone plate, and the plates were incubated 18 hrs at 35°C in a water-saturated incubator. In B several revertants back to the motile, nonchemotactic phenotype are apparent. C. *CheA593*, *mot*$^-$ transduced with P1 from the wild-type strain AW330, showing, away from the inoculum, complete transduction (swarming) and, at the edge of the inoculum, abortive transduction (trails). The conditions of incubation were the same as for A and B.

site of inoculation was usually obtained, probably as a result of the formation of motile but nonchemotactic abortive transductants.

The results, presented in Figure 2, clearly divide the mutants into three complementation groups, although the third is represented by only a single mutant. The three groups have been assigned the genotype symbols *cheA*, *cheB*, and *cheC*. Two double mutants from *cheA593*, one nonflagellated and the other paralyzed, were used in order to show that the nature of the second mutation does not affect the complementation pattern.

The only unusual result was the complementation between the two group A mutants *che-593* and *che-643*. The complementation was not reciprocal, but occurred only with *che-643* as the donor. We have no explanation for this result, but do not feel it warrants classifying *che-593* and *che-643* in separate complementation groups.

Although plates were usually incubated at 35°C, it was found that the yields of trails could be increased by reducing the temperature to 30°C or lower. This phenomenon does not appear to be related to the initial choice between abortive and complete transduction, as holding the plates at room temperature for the first 2 or 3 hrs did not noticeably increase the yield of trails, and incubation at the lower temperature did not appear to reduce the frequency of complete transduction. Perhaps the lower temperature reduces the spontaneous early loss of the abortively transduced fragment, or reduces the chance of lysis of the abortively transduced cell by residual P1.

This effect was exploited in the case of *cheC497*, *fla*$^-$, which usually yielded less than a dozen trails at 35°C; at 30°C the yield was in the range of 20 to 50 trails per plate. A more suitable mutant could not be obtained from *cheC497*.

Complementation with mutants defective in motility: To show that the chemotaxis complementation groups are distinct from those related to motility, defects in which are known to cause paralysis of the flagella (*mot* gene) or altered flagellin (*H* gene), complementation tests were carried out with mutants representing these types. For this purpose paralyzed mutants and a curly mutant that were previously studied by ARMSTRONG and ADLER (1967) were used. Curly mutants have an altered flagellar filament resulting from a mutation in the *H*

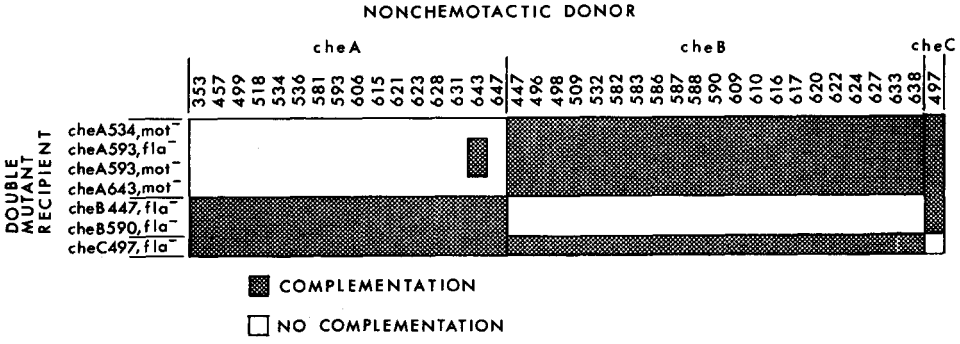

FIGURE 2.—Complementation among nonchemotactic mutants.

gene (IINO 1962), and will not swarm on a semisolid medium since they are unable to carry out translational movement.

The results are shown in Table 1. In all cases complementation was obtained between two mutants with a different phenotype. Thus the genes that control chemotaxis are distinct from the *mot* gene and the *H* gene.

The nonchemotactic-paralyzed double mutants did not complement with all the paralyzed mutants, though their nonchemotactic parents did. This was expected, since previous work by ARMSTRONG and ADLER (1967) showed that paralyzed mutants do not all complement with each other, i.e. they belong to different complementation groups—group I, group II, and an intermediate group. Thus mutants 526, 567, 564 and 625 are known to belong to group I, 507, 524 and 580 to group II, and 260, 458 and 525 to the intermediate group. The mutations leading to paralysis in the three nonchemotactic paralyzed double mutants must be in group II, since they complement with group II but not with group II mutants.

DISCUSSION

The results presented here show that defects in at least three functional units may lead to a loss of chemotaxis toward all substances tried. The genes have been designated *cheA*, *cheB*, and *cheC*. Although *cheC* is represented by only a single mutant, this mutant maps sufficiently away from the *cheA* and *cheB* genes that it clearly belongs to a different gene (J. B. ARMSTRONG and J. ADLER, J. Bacteriol., January 1969). The three *che* genes are distinct from the *mot* gene and the *H* gene.

The nonchemotactic mutants studied in this paper are all *generally nonchemo-*

TABLE 1

Complementation between nonchemotactic, paralyzed and curly mutants

Paralyzed recipient	Nonchemotactic donor *A-534*	*A-593*	*A-643*	*B-447*	*B-590*	*C-497*
260	+	+	+	+	+	+
458	+	+	+	+	+	+
507	+	+	+	+	+	+
526	+	+	+	+	+	+
567	+	+	+	+	+	+
580	+	+	+	+	+	+

Double mutant recipient	Paralyzed donor *260*	*524*	*525*	*564*	*580*	*625*	Curly donor *585*
Nonchemotactic-paralyzed:							
A-534, mot⁻	—	—	—	+	—	+	+
A-593, mot⁻	—	—	—	+	—	+	+
A-643, mot⁻	—	—	—	+	—	+	+
Nonchemotactic-nonflagellated:							
A-593, fla⁻	+	+	+	+	+	+	+
B-447, fla⁻	+	+	+	+	+	+	+
B-590, fla⁻	+	+	+	+	+	+	+
C-497, fla⁻	+	+	+	+	+	+	+

tactic; that is, they do not show chemotaxis toward any substance. In addition, we have now found *specifically nonchemotactic* mutants, which fail to show chemotaxis toward only one chemical or a small group of closely related chemicals and which are considered defective in specific chemoreceptors (G. L. HAZELBAUER, R. E. MESIBOV, and J. ADLER, to be published). The generally nonchemotactic mutants could then be defective in a final common path through which the information from all the receptors is channeled. The existence of three *che* genes indicates that there are at least three steps or three components in this path, but the biochemical lesions resulting from the mutations remain unknown.

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SUMMARY

Complementation tests based on abortive transduction with phage P1 have been carried out with 38 mutants of *Escherichia coli* that fail to carry out chemotaxis toward any substance tried. The results clearly divide the mutants into three cistrons, although the third is represented by only one mutant. These cistrons are distinct from the *mot* gene, which is responsible for the functioning of the flagella, and the *H* gene, which is responsible for the structure of flagellin.

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