mobilities discussed above are not artifacts of the fractionating procedures (*7*) used to prepare ceruloplasmin CH-181, showed that eight of these individual preparations contained at least three ceruloplasmins and two of them contained at least two ceruloplasmins. In view of these experiments on individual sera, it seems probable that the differences in electrophoretic mobility that we have demonstrated in the four ceruloplasmins derived from pooled plasma are due to differences in the content of charged amino acid residues in the native ceruloplasmins. Such a correlation between mobility and amino acid composition has been found for several of the human hemoglobins (*1*). Indeed, our results suggest that ceruloplasmin D_0 possesses a greater number of histidine residues than ceruloplasmin C, since (i) both ceruloplasmins have virtually the same mobility at *p*H 8.5 but ceruloplasmin D_0 is distinctly slower than ceruloplasmin C at *p*H 5.7; (ii) both ceruloplasmins are anionic at each of these *p*H values (*9*); and (iii) histidine is the only charged amino acid residue titrated appreciably between *p*H 8.5 and 5.7 (*14*). On the basis of what is known of hemoglobin, it appears that such differences in structure in ceruloplasmin are likely to be genetic in origin (*15*).

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Isolation of a Bacteriophage Specific for the F+ and Hfr Mating Types of Escherichia coli K-12

Abstract. Seven phage stocks have been prepared from a sample of sewerage. The stocks grow only on the F+ and Hfr mating types of *Escherichia coli* strain K-12. This specificity is not shared by any previously characterized phage which was tested. The specificity of one of the isolates, f1, has been well correlated with the salmonella cross-streak test described by Zinder.

The bacteria *Escherichia coli* have been shown to consist of three mating types: F−, F+, and Hfr (*1, 2*). F− strains serve as recipients for genetic material donated by F+ or Hfr strains during conjugation. F+ and Hfr strains differ in two respects: First, under proper selective conditions Hfr strains display a higher frequency of recombination with F− strains than do F+ strains. Second, F+ strains possess a highly infective F agent capable of converting F− strains to the F+ mating type. This agent is not detectable in Hfr strains. Thus, matings of Hfr and F− strains result in F− progeny (or Hfr progeny under certain conditions), while the progeny of F+, F− matings are F+. It is possible to eliminate the F agent from F+ strains with chemical agents, thus converting these bacteria to the F− mating type (*3*).

There are also differences in the physiology of F+ (or Hfr) and F− bacterial cells. F+ cells show a greater tendency to autoagglutinate, agglutinate at a higher *p*H, and are more easily stained with acid dyes than the corresponding F− cells (*4*). Furthermore, Zinder has described a "staining" interaction between *E. coli* and *Salmonella typhimurium* and between *E. coli* and *E. coli* which distinguishes F− from F+ or Hfr bacterial mating types (*5*).

In view of the above observations, it seemed reasonable to search for a bacteriophage capable of distinguishing F− from F+ and Hfr mating types. This report describes the isolation and testing of such a phage.

The following mutant strains of *E. coli* K-12 were used [prepared as described by Lederberg (*1*)]: 58-161 (F+), Hfr (Cavalli), and Y-10 (F−). The pertinent characteristics of these strains are as follows: 58-161 and Hfr (Cavalli) are capable of lactose fermentation (lac+) but are unable to grow in the absence of methionine (meth−); Y-10 is incapable of lactose fermentation (lac−) and requires threonine, leucine, and thiamine for growth (thr−, leu−, thi−). Two strains of salmonella were used: *S. typhimurium* LT 2 and LT 2 F+ (LT 2 infected with the F agent of *E. coli* K-12 (*5*). Other bacterial strains which were used are described in the text where appropriate. Various bacteriophages were used and are described in the text where appropriate.

The media used have been described by Lederberg (*1*). In particular broth agar, minimal agar, and eosin methylene blue agar with lactose (EMB lac) and without sugar (EMB 0) were employed.

Phage plating was done by the method of Adams (*6*). Also cross-streaking of bacteria with phage on EMB 0 provided a sensitive test for bacterial susceptibility.

The mating type of a bacterial strain was determined by mixing the strain with F− bacteria auxotrophic for nutrients complementary to the deficiencies of the strain being tested. The production of colonies by this mixture when spread on minimal agar indicated the mating type to be either F+ or Hfr (*1*).

Mating types were also determined by cross-streaking against *S. typhimurium* LT 2 on EMB 0 following the method of Zinder (*5*).

In order to isolate a phage with the required characteristics it was necessary to obtain two strains of *E. coli* K-12 identical in all respects but mating type. To accomplish this it was decided to "cure" an F+ strain by the method of Hirota (*3*). Acriflavine was added to a saturated broth culture of 58-161 (F+) to a final concentration of 1 mg/ml. After 2 hours at 37°C, the culture was washed and streaked on broth agar. A clone (labeled 58-161 F−) was isolated which was incapable of colony production when mixed with Y-10 (F−) on minimal agar (*1*). Clones 58-161 and 58-161 F− were found to be identical in all respects other than mating type.

To test the possibility that some previously isolated phage would distinguish mating types, the following phages were spotted on plates seeded with 58-161 or 58-161 F−: T1, T2, T3, T4, T5, T6, T7, C16, lambdal, P22, P22V, phi, P2, and megatherium T. An identical pattern of susceptibility and resistance was displayed by the two strains.

To isolate and characterize a phage that grows only on F+ and Hfr strains of *E. coli* K-12, sewer water was centrifuged and sterilized with chloroform; nutrient broth was added to preserve phage. Forty-eight plaques were isolated from a plating of this solution on 58-161 F− and 90 plaques from plates seeded with Y-10 (F−). The spotting of these

isolates on 58-161 and 58-161 F− produced an identical pattern of susceptibility and resistance. Twenty-four plaques were isolated from a plating of sewer water on 58-161 (F+) and 90 plaques from plates seeded with Hfr (Cavalli). Seven of the isolates spotted on 58-161 but not on 58-161 F−. Stocks were prepared from these isolates (labeled f1, f2, and so on), and each was titrated on 58-161 (at no dilution did plaques appear on 58-161 F−).

Although enough experiments have been performed with each of the above stocks to assure that they all distinguish F+ from F− strains, the following tests refer to one stock only, f1.

To determine the sensitivity of various bacteria to f1, f1 was cross-streaked on EMB 0 agar against 21 strains of *E. coli* K-12 of various genotypes and mating types. Each of seven F− strains tested was resistant, whereas nine F+ and four Hfr strains were sensitive. A derivative of Hfr (Cavalli) mating less efficiently than the parental strain was resistant to f1, an exception also demonstrated by cross-streaking against *S. typhimurium*. Cross-streaks against the following strains of *E. coli* were all negative: *E. coli* B, *E. coli* Raper, *E. coli* Wilson, Wg 35 A, W 52 A, and Wg 4A (N8) [the latter three coli strains were isolated by Bernstein (*7*)]. Cross-streaks against the following bacteria were also negative: *S. typhimurium* LT 2, LT 2 F+, *Shigella sonnei, Shigella dysenteriae*, and *Aerobacter aerogenes*.

The f1 phage appears to be highly specific for the F+ and Hfr mating types of *E. coli* K-12. This notion is supported by the following experiments.

1) The first experiment was concerned with the transfer of F from *E. coli* to *E. coli*. F− bacteria become F+ when grown in mixed culture with F+ strains. On the other hand, F− strains remain F− when grown with Hfr bacteria. In either case the original F− population can easily be reisolated by using some sugar fermentation as a differential marker. This experiment offers an excellent opportunity to demonstrate that sensitivity to phage f1 is correlated with the F+ mating type.

After growth of Y-10 (F−, lac−) with either 58-161 (F+, lac+) or Hfr (Cavalli) (lac+), the mixtures were diluted and plated on EMB lactose agar. Twenty-one lactose-negative colonies were isolated from the 58-161, Y-10 plates; these were f1 sensitive and F+ (as shown by an ability to mate with the appropriate F− strain). Ten lactose-negative colonies were isolated from the Hfr (Cavalli), Y-10 plates; these were f1 resistant and F− (demonstrated as above).

2) The second experiment was concerned with the transfer of F from *S. typhimurium* to *E. coli*. The design of this experiment was exactly like that of the coli to coli F transfer. LT 2 F+ and Y-10 (F−), two strains resistant to f1, were mated. One hundred clones were isolated and tested for mating type by cross-streaking against *S. typhimurium* (*5*). Of these isolates, 18 selected clones were also tested for susceptibility to f1. Three of these gave positive cross-streaks against f1 and *S. typhimurium*, and 15 gave negative cross-streaks in both tests. Thus, the phage test and salmonella test are perfectly correlated (*8*).

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Esters Produced by *Chalaropsis thielavioides*

Abstract. Chalaropsis thielavioides, a filamentous fungus, produces a characteristic ester-like aroma on potato dextrose broth. By gas-liquid chromatographic techniques, the major aroma-producing substances have been identified as isobutyl and ethyl acetate. Lesser amounts of isoamyl acetate, ethanol, and methanol were also present.

The formation of esters by filamentous fungi has not been extensively investigated. Gordon (*1*) reported the production of ethyl acetate by *Endoconidiophora moniliformis. Penicillium digitatum* also synthesizes ethyl acetate (*2*). The formation of isobutyl acetate and methylheptenone by *Endoconidiophora coerulescens* has been reported by Morgan (*3*). Other esters synthesized by filamentous fungi include methyl-*p*-methoxy cinnamate (*4*, *5*), methyl cinnamate (*4*), and methyl anisate (*6*). Longree (*7*) noted an odor resembling that of isobutyl acetate emanating from her cultures of *Chalaropsis thielavioides*. Her observation, however, was not confirmed by analysis of the culture volatiles.

In the spring of 1958 *C. thielavioides* Peyrn. was isolated from dogwood graft failures. During the initial study it was noted that cultures of this organism produced a characteristic ester-like aroma. The purpose of the present study was to identify the volatile constituents responsible for the aroma.

On culturing the organism in various natural media, the production of the characteristic aroma appeared to be best in potato dextrose broth cultures shaken continuously during incubation. Therefore, 25 250-ml erlenmeyer flasks each containing 75 ml of this medium were inoculated and incubated on a shaker at room temperature until a strong aroma could be detected emanating from the flasks. This generally took from 4 to 5 days. The cultures were then pooled aseptically in a 5-lit. round-bottom flask, and this was connected in a gas-train apparatus. The train included flasks for sterilizing and moistening the air before passing it over the cultures and a series of four traps, one cooled with wet ice and alcohol and three with dry ice and alcohol, for condensing the volatiles removed from the cultures.

In different trials air was passed over the cultures for periods varying from 18 to 40 hours. The cultures were agitated during aeration by a magnetic stirring bar. During the longer collecting periods, several milliliters of condensate reached the second dry-ice-alcohol trap and appeared as two layers. In some experiments the condensate was extracted with ether, and the ether was removed by careful distillation; in others, drying the volatiles over anhydrous sodium sulfate provided sufficient material for satisfactory analysis.

Table 1. Gas-liquid chromatographic analysis of the volatiles produced by *Chalaropsis thielavioides* in potato dextrose broth.

Compound	Retention time				Approx. percentage of total
	Carbowax 1500*		Di-*n*-decylphthalate†		
	Known	Volatile	Known	Volatile	
Acetone	1′ 14″	1′ 13″	1′ 10″	1′ 10″	(Trace)
Ethyl acetate	1′ 46″	1′ 46″	2′ 7″	2′ 7″	30
Methanol	2′ 27″	2′ 27″	40″	40″	2
Ethanol	3′ 1″	3′ 2″	1′ 2″	1′ 2″	5
Isobutyl acetate	3′ 56″	3′ 56″	6′ 41″	6′ 43″	58
Isoamyl acetate	7′ 48″	7′ 43″	14′ 36″	14′ 34″	4
Isobutyl alcohol	7′ 55″	‡	3′ 48″	3′ 48″	(Trace)
Isoamyl alcohol	17′ 8″	§	8′ 54″	8′ 58″	(Trace)

* Perkin-Elmer K column; length, 2 m; temperature, 80°C; gas, helium; pressure, 20 lb/sq. in.; flow rate, 46.5 ml/min; sample, 2 μl; sensitivity, 10 mv. † Perkin-Elmer A column; length 2 m; temperature, 100°C; gas, helium; pressure, 20 lb/sq. in.; flow rate, 50.5 ml/min; sample, 5 μl; sensitivity, 10 mv. ‡ Isobutanol not separated from isoamyl acetate. § Trace of isoamyl alcohol not detected.