B and produces some rings spontaneously. The conidia of these three types are morphologically indistinguishable and can only be separated according to their ability to form rings spontaneously. Thus a certain percentage of apparently B type conidia are actually A/B type conidia which will produce both A and B type responses when germinated.

These data indicate that the genetic control of ring formation in the predaceous hyphomycete *Dactylella doedycoides* Drechsler is a heterocaryotic phenomenon. The ability of this fungus to produce constricting rings in the presence of free-living nematodes may be quite variable because of the nuclear heterogeneity indicated by the data presented here. These data explain many of the anomalous observations which have been recorded in the literature concerning ring formation in the predaceous nematode-trapping hyphomycetes (*5*).

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Sexuality and Mating in Salmonella

Abstract. Another criterion for the presence of the agent (F) promoting genetic exchange in *Escherichia coli* was found. It involves a staining reaction of Hfr and F+ coli, but not F− coli, when mixed with strains of *Salmonella typhimurium*. This reaction was used as a guide in following the transfer of the F agent to *Salmonella* and back to *E. coli*. The F agent in *Salmonella* seems to promote the same kinds of events that it promotes in *E. coli*.

The mating type system in the bacteria *Escherichia coli* has been shown to be under the control of an agent called F (*1, 2*). Those bacterial strains that lack F act as recipients for genetic material, while strains that have F act as donors of genetic material. It has been concluded from the work of Wollman and Jacob (*3*) that the genetic material of the donor passes into recipient bacteria in a linear fashion. There is no evidence for the reciprocal transfer of genetic material from recipient bacteria to donor bacteria (*4, 5*).

The F agent can exist in at least two states in donor strains. One state confers upon donor bacteria the ability to pass the F agent to recipients at high frequency, but the chromosome at low frequency (F+ bacteria). The other confers the ability to donor bacteria of passing their chromosome (or part) at high frequency, while the F agent is transferred at low frequency (Hfr bacteria). The transmission of the F agent in the former case results in an F+ bacterium, while in the latter it results in an Hfr bacterium. It has therefore been inferred that the difference between these two kinds of bacteria depends upon the localization of the F agent: F+ bacteria have the agent in a free state, while Hfr bacteria have the agent at a particular one of a number of possible chromosomal sites. It has also been inferred that all effective transfers of genetic material from F+ to F− bacteria involve either a transient or permanent fixation of the F agent to the chromosome (*6*).

One other bacterial property might be ascribed to the F agent. Since, for an effective mating, the two mating types must make a sufficient cell-by-cell bridge, some differences in the surface properties might be expected. Differences in the staining and agglutinating properties have been observed (*7*). Thus, it would seem that F− bacteria change their surface when "infected" with the F agent. In this respect the action is analogous to that produced by some temperate bacteriophages, a process called lysogenic conversion (*8, 9*). Loeb (*10*) reports a bacteriophage which will differentiate F+ and Hfr bacteria from F− bacteria.

Baron *et al.* (*11*) and Miyake and Demerec (*12*) have shown that some strains of salmonella will act as recipients for genetic material from Hfr coli. Since Zinder and Lederberg (*13*) tested many of these same salmonella strains for mating *inter se* and failed to find any evidence for this, it might be assumed that salmonella is, as is the average coli, F−. Therefore, if the F agent could be transferred to salmonella, they too might mate. This report describes the successful transfer of the F agent to salmonella and the properties of the resulting F+ organisms.

The preparation of mutant lines of bacteria was accomplished as described by Lederberg (*14*). The abbreviations used to describe the genotypes are as follows. The fermentation mutants are designated "lac" for lactose, "gal" for galactose, "arab" for arabinose, "mal" for maltose, and "mtl" for mannitol.

Drug resistance mutations are as Az^r for resistance to sodium azide. Auxotrophic mutations and notations used are "meth" for methionine, "pro" for proline, "thr" for threonine, "leu" for leucine, "try" for tryptophan, and "his" for histidine.

The derivatives of *E. coli* K-12 that were employed were Hfr (Cavalli) (*1*) of genotype meth−; Hfr (Hayes) (*15*) of genotype meth− Az^r; F+ and F− with genotypes for both of meth− and thr−, leu−, lac−, gal−.

The salmonella strains were all derivatives of *S. typhimurium* LT2 (*13*). Their genotypes are described in the text.

The special media for the study of bacterial genetic processes were the same as those described by Lederberg (*14*). Of special use in this study was eosin methylene blue agar without any sugar supplementation (EMB 0).

In *E. coli* F− organisms become F+ during mixed culture with F+ organisms. This can be demonstrated by reisolating the orginally F− organisms on the basis of some differentiating marker and testing their mating behavior. Since coli are lactose fermenters and salmonella are nonfermenters, the reisolation of the salmonella type after mixed culture with coli is readily accomplished. However, the recognition of any transfers of the F agent is more difficult. It would involve the testing of a large number of independent isolates either for ability to transfer back to *E. coli* F−, the F agent, or for mating with other salmonella strains. Both of these are contingent properties, in that F might be transferred to salmonella with very low frequency and similarly have a low, if existent, frequency of back transfer, or that it might not, in salmonella, confer mating ability. Of some use in this and other problems would be some other criterion for the F state of bacteria.

In the course of some experiments, it was noted that when an F+ or an Hfr coli was cross-streaked with LT2, or some other salmonella, on EMB 0, there would be a straining of the bacteria in the area of their intersection: a red spot. Such reddenings are characteristic of the cross-streaks of lysogenic and nonlysogenic bacteria. The lysis of the nonlysogenic bacteria after attack by the phage released from the lysogenic bacteria releases some acid, thereby lowering the *p*H and allowing eosin to stain. Eosin stains bacteria at *p*H below 5.5 and hence is used in the EMB medium to determine fermentative responses. However, no evidence for any propagable entity such as phage was found.

An effort was made to correlate in detail the staining reaction and the F status of the coli. An F+ and an F− coli were grown in mixed culture, and on the basis of some independent marker, such as lactose fermentation,

clones of the F— type were isolated. Twenty such clones were obtained and were mated with the appropriate F— coli. Twelve of the clones gave recombinant progeny and therefore had been converted to F+. The same 12 clones gave a positive staining reaction when cross-streaked with salmonella. In a similar experiment with the mixed growth of Hfr and F—, none of the 20 F— clones that were isolated scored as F+ by the mating test, and none gave the staining reaction.

The same pairs described above were mated. By using the same set of selective markers in each cross, the same general classes of recombinant types would be obtained. The F+ and the Hfr parent were both meth—, while the F— parent was thr—, leu—. The selection was for independence of these growth factors. All but one of the 20 tested progeny from the F+ cross gave the staining reaction when tested with salmonella; the progeny of an F+ by F— cross are primarily F+ (*1*). None of the progeny of the Hfr cross gave the straining reaction when tested with salmonella; the progeny of Hfr by F— crosses are primarily F— (*1*).

One further test was available. F+ bacteria can be cured of the F agent by treatment with acridine dyes (*16*). One such strain, prepared by Loeb (*10*) was F— by mating test and also by staining reaction. When it was reinfected with the F agent both properties were restored.

The nature of the staining reaction is not clear, and as currently accomplished it is difficult to quantitate. There is no evidence that any diffusible substance is involved, for the staining is restricted to the overlap areas. In order for it to permit adequate scoring, the two bacterial cultures must be inoculated at approximately equal densities, and the final growth must be confluent. It is not necessary that it be accomplished on EMB medium. When the appropriate bacteria are grown together on a nutrient agar and this growth is scraped off and the bacteria are suspended in buffered eosin, the cells stain at a *p*H as high as 7.0. When the stained bacteria are observed microscopically, the stain forms a polar granule, giving the organisms an appearance not unlike that of sporulating bacillus. The salmonella and coli look quite different, and therefore it is certain that the salmonella are stained; some but not all of the coli are also stained. As far as can be determined the cells are all viable. There is no evidence for killing of salmonella by coli cells or vice versa, for mixing known numbers of the two organisms and determining, in time, their relative number show no change. The phenomenon would therefore seem to involve some surface-to-surface interaction which causes sufficient damage to allow eosin to penetrate the cells and stain the acid cytoplasm. In this connection it is interesting to note that rough salmonella (devoid of somatic antigen) stain readily with eosin.

None of several different F+ coli interact with F— coli. Of the two Hfr available here, only Hfr (Cavalli) interacts with F— coli, and it does so somewhat erratically. All of five different wild type strains of *S. typhimurium* interact with F-carrying coli. There are two general impressions; first, that F— bacteria recently converted to F+ interact more strongly than older cultures, and second, that Hfr gives a more intense reaction than the F+ bacteria.

From the stained areas obtained by mixing F+ coli with LT2 derivatives, the bacteria were streaked out and the salmonella were tested for staining reaction, as it was hoped that an F+ salmonella would be "immune" and would not interact. Of 180 individual colonies tested, two did not give the reaction with F+ or Hfr coli. By all other criteria these bacteria were salmonella. They gave the typical reactions with salmonella antisera, both flagellar and somatic. They had the characteristic salmonella fermentation pattern and phage sensitivities. These strains were cross-streaked with F— coli and although there was no staining reaction, the coli were isolated. A total of three of 100 tested gave positive responses with wild-type salmonella, and these same three mated well with the appropriate F— coli. Thus, the F agent had been passed to salmonella and back to coli and had retained its capacity to confer mating ability and the staining reaction. Similar mixtures of the F+ salmonella with other differentiable salmonella strains gave transmission frequencies of the F agent of 25 to 75 percent (loss of reactions with Hfr coli), although no staining reaction occurred with these mixtures.

Mating of Salmonella. 1) F+ by F—. One of the presumptive F+ strains was a meth—, his—, gal— derivative of LT2. It was mated, by mixed growth, with a number of different auxotrophs such as a pro—, a try—, and a leu—. In all instances prototrophs appeared with a frequency of about 1 in 1 million of the parental cells. The gal marker segregated among these progeny. The same crosses were done with the parent culture of the F+ salmonella. Prototrophs appeared only at the lower frequencies characteristic of the reversion for the individual markers.

Crossing the F+ strain with a cys— arab— mtl— mal— gave many prototrophic recombinants of the unselected classes. The data are insufficient as yet to permit presentation of detailed linkage maps, but they are indicative of linkages extending over fairly large segments of chromosome, the criterion by which recombinational and transductive phenomenon are most readily distinguished.

F+ strains have been prepared from a number of different auxotrophs, and in all instances they gave prototrophic recombinants when mixed with other auxotrophs.

2) Hfr by F—. Many of the difficulties which were originally encountered in the genetic mapping of *E. coli* have been explained by the assumption that all transfers in a mating event occur through the intervention of Hfr bacteria (*5*). Since the same F+ can give rise to different Hfr, the map obtained in F+ by F— or F+ by F+ crosses would be a composite of different kinds of events and except for very small regions be nonlinear. Therefore, before proceeding further with the genetic analysis of salmonella, it was necessary to determine whether Hfr salmonella existed and could be isolated.

Hfr bacteria were sought by the replica plating and indirect selection technique of the Lederbergs (*17*). This procedure, which first was used for this purpose by Jacob and Wollman (*6*), involves growing of F+ bacteria on a petri dish containing a nutrient medium and stamping this growth on a selective medium which had been previously spread with a large number of F— bacteria. Recombinant colonies on the selective medium would determine the site of any Hfr mutants on the original F+ template. This area is then picked and since it represents only a fraction of the original template thereby increases the concentration of Hfr cells relative to the total. The process is then repeated at a lower dilution of plated organisms, which allows for bigger individual clone sizes (the medium limits the total possible growth). Eventually, a pure clone can be obtained. The technique described above gave primary enrichment for Hfr, but no enrichment on further transfer. There was no doubt, however, that many of the recombinants were formed from particular clones of the F+ parent. When the F+ was irradiated with ultraviolet light to give about 10^{-4} survivor, prior to plating for the original template, relatively stable Hfr could be isolated. About 10^4 survivors were plated on individual petri dishes and, since the medium supports the growth of more than 10^{11} organisms, any Hfr induced by irradiation would be in a clone of about 10^7 cells. Since its frequency among the population on the particular plate would be about 1 in 10^4 and the average enrichment obtained by picking congruent sites is 100-fold, the Hfr is readily isolated in the next cycle. Two different Hfr strains

have been isolated by using different combinations of selective markers. They are being analyzed further.

With regard to the staining reaction, neither the Hfr nor F+ salmonella give the reaction when mixed with F− strains, either salmonella or coli.

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Suppression of Radiation-Induced Tumorization in Fern Prothalli

Abstract. By quantitative techniques it has been shown that the expected tumor frequency after x-irradiation of spores of the fern *Pteridium aquilinum* can be reduced by the addition of casein hydrolyzate or amino acids to the medium upon which the spores are germinated and grown into either prothalli or tumors.

To analyze the process of tumorization and to attempt to characterize it in terms of fundamental cellular processes, it would seem advantageous to study the phenomenon in the simplest biological system in which it can clearly be recognized. One such system, which is perhaps unique in many ways, is that of the prothallial or gametophytic generation of the fern (*1*). In *Pteridium aquilinum* (the bracken fern) a readily recognizable tumorization occurs spontaneously at a very low rate, but the frequency can be increased in response to ionizing radiations (*2*). Although certain criteria for the word *tumor* are inapplicable here, since *tumor* was originally defined in the frame of reference of more complex biological systems, these abnormal growths are considered to be tumors in that they are unlimited, disorganized growths characterized by many cellular changes typical of tumors in higher organisms. At least in the lower dose range, the response to ionizing radiations is linear, and the spontaneous frequency is increased by approximately 100-fold at 15,000 r of x-rays applied to dormant spores. This process can be quantified quite precisely (*3*). The primary causal event to subsequent tumorization is known to occur in a single haploid cell, the spore. The spores and the resultant prothalli and tumors are germinated and grown aseptically in vitro on chemically defined media.

To study factors involved in tumorization in this system, experiments were performed as follows (*4*): Spores of *Pteridium* in the dormant condition were irradiated at 1000 r/min of unfiltered 136-kv x-rays for a total dose of 15,000 r. The spores were then sterilized and sown either quantitatively (*3*) into petri plates, or semiquantitatively onto agar slants in 25-mm culture tubes. The quantitative technique was used to assay possible effects on viability, whereas the more rapid semiquantitative technique was used for preliminary and large-scale screening. In this latter method, a Carbowax suspension of spores was made as for the fully quantitative method, but with a higher concentration of spores (100 mg/cm^3). Then quantitatively comparable inocula were taken for the control and experimental lots, without making actual spore counts, by dipping a suitable wire inoculating loop into the spore suspension and streaking this onto the slanted agar surfaces. It has been found that the drop which fills the loop contains approximately identical inocula in repeated immersions from one spore suspension.

To assay for the effects of additions to, or alterations of, the medium, these quantitatively similar inocula of x-irradiated spores were streaked onto the control, or basal, medium and onto the experimental media, in rotation. The basal medium is essentially a Knudson's mineral salt solution with added minor elements, sugar and agar (*1*); in all experiments here described spores were grown on 0.25 percent sucrose and 1.5 percent Difco Bacto-agar. This selection of a basal medium was purely arbitrary; it was an outgrowth of earlier work with the culture of fern prothalli. Since the discovery of the phenomenon of induced tumorization and all earlier quantitative work had been on this medium, it was used as a point of departure for subsequent work. Thus, in all experiments the "basal" tumor frequency is defined as the frequency of tumors for each individual experiment on this basal or control lot. Experimental lots are then expressed on a percentile basis as compared with the control lot. The experimental lots have either this basal medium plus an added substance, or, in later experiments, a modification of the basal medium. All recognizable tumors were counted at the end of 6 weeks in culture.

Table 1. Frequency of tumors in prothalli of *Pteridium aquilinum* on various media after x-irradiation of dormant spores, expressed as percentage of frequency on basal medium.

Addition to basal medium	Tumor frequency (%)
Casein hydrolyzate, 2.5 ml/lit. (see *5*)	42
Casein hydrolyzate, 5.0 ml/lit.	34
Synthetic casein hydrolyzate (total)*	34
Monoaminomonocarboxylic amino acids	52
Hydroxyamino amino acids	40
Sulfur-containing amino acids	35
Benzenoid amino acids	42
Basic amino acids	82
Acidic amino acids	96
Pyrrolidyl amino acids	40
NH_4NO_3, 680 mg/lit.	109
NH_4NO_3, 1360 mg/lit.	116

* Our synthetic approximation of casein hydrolyzate, compiled from amino acids obtained from Mann Research Laboratories, Inc., New York, was made up as follows, in milligrams per 100 ml of stock solution, used at the rate of 100 ml/lit. of medium: monoaminomonocarboxylic: glycine (20), L-alanine (35), L-valine (70), L-leucine (100), and L-isoleucine (60); hydroxyamino: L-serine (60) and L-threonine (45); sulfur-containing: L-cystine (5) and L-methionine (30); benzenoid: L-phenylalanine (50), L-tyrosine (60), and L-tryptophane (20); basic: L-lysine (70), L-arginine monohydrochloride (50) and L-histidine monohydrochloride (30); acidic: L-aspartic acid (70) and L-glutamic acid (220); pyrrolidyl: L-proline (80) and L-hydroxyproline (20).

With this experimental approach, first various plant growth hormones (auxins) were tested, but with no apparent effect. This emphasized the possible magnitude of the task if future testing were to be done with specific substances. Thus, in an attempt to test broader categories of substances, casein hydrolyzate (*5*) was among those tested. This was found to depress the incidence to as low as 34 percent of the basal rate in the concentrations used. For further information on this and other results discussed, see Table 1. Several replications confirmed this initial observation. Next, to determine whether this effect was due to the amino acids, a synthetic approximation of casein hydrolyzate was made, based upon published analyses of casein (*6*). This synthetic "casein hydrolyzate" was found to have a similar tumor-inhibiting effect, depressing the incidence to the lowest value observed with the original hydrolyzate. Next, the component amino acids were arbitrarily categorized as to structural types, and these groups were tested separately, in the same concentrations in which they occurred in the total mixture. The effectiveness of the groups varied, and no one group was quite as effective as the total, but the results indicated that the effectiveness of the total mixture was due to several of the component amino acids, and also that the action of the various amino acids was more