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Surface Properties Correlated with Sex Compatibility in *Escherichia coli*

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SUMMARY: Sex incompatible (F⁻) strains of *Escherichia coli* have a lower acid agglutination point, a stronger affinity for a basic dye and a greater stability in broth cultures as compared with F⁺ strains of corresponding phenotype. These findings indicate a correlation between some surface properties and sex compatibility. These surface properties and the F⁺ state are transmitted infectively from F⁺ to F⁻ strains at the same time. Screening for cultural behaviour of infected strains yields results fully consistent with screening for sex compatibility.

In the last few years evidence has been offered for a system of sexual compatibility in *Escherichia coli* K12 (Lederberg, Cavalli & Lederberg, 1952; Cavalli, Lederberg & Lederberg, 1953; Hayes, 1953). On this ground it has been possible to distinguish between self-compatible strains (termed F⁺) defined as those which are productive of recombinants even when crossed with incompatible strains (termed F⁻) which, in turn, have been defined as those which recombine only with F⁺ strains. The property F⁺ is transmissible from F⁺ to F⁻ organisms by *infection* due to an agent (F) which has not yet proved separable from the organism.

The nature of F is still unknown mainly because no physiological changes have been so far correlated with changes in F state. However, the great amount of data collected by the authors quoted above does contain useful indications for further investigations in this direction.

Infection seems to be effected by a short contact between single organisms of opposite F state, which modifies properties determining frequency of recombination both relatively and absolutely. In fact, F⁺ × F⁻ crosses are more productive than F⁺ × F⁺ ones. The far lower frequency of recombination, compared with that of infection, together with the fact that all recombinants from F⁺ × F⁻ crosses are F⁺, suggests that infection is a prerequisite of recombination.

On the other hand, F⁺ and F⁻ seem to be characters resulting from a different degree of the same property, on the ground that in a cross F⁺ × F⁺ one of the two strains behaves mostly as F⁺ and the other as F⁻.

These data, if considered all together for a coherent interpretation, focus the problem of sex compatibility on the factors which determine the contact of organisms. Tentatively, it might be thought that some sort of interaction within the surface of organisms defines the probability and the extent of their contact, besides such other obvious factors as density, agitation, etc., of the suspensions of organisms.

It seems worthwhile, therefore, to search for differences in surface properties between organisms of opposite F state, especially since the finding of properties correlated with sex compatibility might offer new experimental criteria for investigating the problems outlined above. In fact, the screening of F+ and F- strains has been concerned, up to now, only with testing their capacity to recombine with strains of known F state and with ability to transmit F by infection. Both methods, apart from the obvious merit of yielding a direct answer, have the disadvantage of being unsuitable for large-scale experiments.

A comparative study of some surface properties of F- and F+ strains has been made by determining: (1) the acid agglutination point; (2) the affinity for acid and basic dyes at different pH values; (3) the stability of broth cultures.

The preliminary results of this investigation (Maccacaro, 1955) were such as to suggest further work of which this paper is the report.

METHOD

Organisms. The strains of *Escherichia coli* used in this investigation were all derivatives of *E. coli* K12 and were kindly supplied by Dr L. L. Cavalli Sforza and Dr L. Fischer Fantuzzi. The phenotype of the strains is fully described in Table 3. Eight of the strains were used more often than the rest, thus permitting factorialization of: (1) two groups of markers, including biochemical deficiencies, sugar fermentations and virus resistance; (2) sensitivity and resistance to streptomycin; (3) F- and F+ state, according to Table 1.

Table 1. *Phenotype and mating type of the 8 strains of Escherichia coli K12 most often used in this investigation*

For the meaning of symbols see Table 3.

Determination of the acid agglutination point (a.a.p.). This was carried out by suspending twice-washed organisms in buffers of values ranging from pH 2 to 5 by 0.2 intervals. Buffers were made from 0.1 M-citric acid and 0.2 M-disodium hydrogen phosphate mixed in suitable proportions. Final suspensions contained about 5×10^7 organisms/ml.

Affinity for acid and basic dyes was tested (Tolstouhov, 1929) by using a mixture of 1 vol. 3.2% (w/v) 'Methylenblau B extra Merck' in pure methanol as basic dye, and 10 vol. of 0.5% (w/v) 'Rubin S extra konzentriert Merck' in pure methanol as acid dye. By addition of 0.1 N-HCl, samples of this dye solution ranging from pH 2 to 5 were prepared and used to stain smears of organisms.

Stability of broth cultures was tested nephelometrically in a Zeiss Stufenphotometer, with conical beam of light and filter L1. The medium was either the infusion broth prepared by the Istituto Sieroterapico Milanese (500 g. horse muscle + 20 g. peptone + 5 g. NaCl in 1000 ml. distilled water), or our nutrient broth NB5 (Beef extract Difco 10 g.; Bacto Peptone Difco 10 g.; Nutrient Broth Difco 8 g.; glucose 5 g.; NaCl 8 g. distilled water to 1000 ml.). Both media were adjusted to pH 7.3 and adjusted to the same optical density. Nephelometrical readings were taken, if not otherwise stated, at the 20th hour of incubation.

Infection was carried out by mixing the F- and the F+ strains in a large-size test tube containing 5 ml. of one of the two broth media mentioned above. The test tube was then placed in a water bath at 37° and gently shaken throughout the time of infection. When the two strains had been chosen for opposite fermentation marker characters, their subsequent isolation was always made on EMB medium (Lederberg, 1950).

Recombination was carried out by inoculating 10^8 – 10^9 washed organisms of each strain on the surface of minimal agar plates (medium of Lederberg, 1947) occasionally supplemented with thiamine.

RESULTS

Determination of the acid agglutination point

The determination of the acid agglutination point (a.a.p.) was repeated four times for each of the 8 strains studied. Mean values for single strains and for one-factor groups of them are given in Table 2. The statistical analysis of these data shows that the factor most affecting a.a.p. of the studied strains was, by far, the F state ($P < 0.01$). Streptomycin resistance had no effect on a.a.p. ($P > 0.05$), while the effect of the other markers was just significant ($0.05 > P > 0.01$) in the sense that, in terms of a.a.p., M_1 strains were to M_2 as, broadly speaking, F- strains are to F+. No interaction between these effects is significant.

Table 2. *The acid agglutination point (a.a.p.) of 8 strains of Escherichia coli K 12*

The characters of strains are indicated by the headings of the columns and rows. The datum is the pH value at which agglutination occurs.

	M_1	M_2	
F- { St ^s	3.000 ± 0.041	2.925 ± 0.050	2.913 ± 0.035
{ St ^r	2.775 ± 0.087	2.950 ± 0.065	
F+ { St ^s	3.350 ± 0.105	3.450 ± 0.076	3.331 ± 0.059
{ St ^r	3.100 ± 0.032	3.425 ± 0.112	
	3.056 ± 0.066	3.188 ± 0.075	

Figures are means and standard errors of four replicates. Marginal means refer to the corresponding one-factor quartet of strains.

The mean a.a.p. for St^s strains is 3.181 ± 0.068 versus 3.063 ± 0.074 for the St^r strains.

For the meaning of symbols see Tables 1 and 3.

Affinity for acid and basic dyes at different pH values

Staining of the 8 strains with a mixture of Methylenblau B and Rubin S at different pH values resulted in a differential retention of the two stains by the organisms, varying from exclusively Methylenblau B to exclusively Rubin S, according to shifting of the pH values from 4 to 2. The data collected are not yet adequate for a satisfactory analysis, because of some difficulties in standardizing the experimental conditions. Nevertheless, it can be safely said that for any given pair of the studied strains it was possible to find a zone of pH values within the range pH 4–2, at which the F+ variant retained the acid stain, while the F– variant retained the basic one.

Stability of broth cultures

The stability of broth cultures of strains with opposite F state was studied nephelometrically. Provided that the light beam impinged on the test tube at about 2 cm. from the bottom, the nephelometer readings were not a function of the total number of organisms in the culture but of the number of them which remained suspended in the medium. In fact, comparable cultures of pairs of strains, identical but for sex compatibility, when shaken gave comparable nephelometer readings and viable counts. As seen from Fig. 1, both F+ and F– strains grew at the same rate as indicated by the nephelometer readings, until a population density was reached at which F+ organisms precipitated while F– organisms did not.

Fig. 1. Broth culture stability of 3 F+ strains (black marks) and 3 F– strains (white marks). Medium: infusion broth I.S.M.

Curves given in Fig. 1 are samples chosen from many others and do not represent an extreme situation. On the contrary, a far greater difference between end-points was found very often. It is worth noting that as a rule such curves show some periodicity, just as if the spontaneous precipitation, by clearing the medium, allowed further growth of suspended organisms up to the concentration at which precipitation recurred.

Fig. 2. Interclonal variability of broth culture stability of 8 strains. Shaded histograms relate to F+ variants. Suspension turbidity is recorded in abscissa and decreases continuously from left to right: for sake of simplicity the abscissa values are untransformed readings on the two scales of the Zeiss Stufenphotometer.

Medium: infusion broth I.S.M.

Curves given in Fig. 1 relate to cultures in infusion broth produced by the Istituto Sieroterapico Milanese. They are not reproducible in a poor medium like Nutrient Broth Difco, unless this is suitably supplemented, as is our nutrient broth NB5.

Table 3 shows the effect of the F state on the stability of broth cultures of 17 pairs of strains with very different phenotypes. The data are recorded as the logarithms of nephelometer readings; it is apparent that for any given pair of strains, the F- strain scores regularly higher, no matter what the phenotype.

However, the extent of such a difference is by no means constant from strain to strain nor, within each pair of strains, is it constant over the two media used. It is our experience that for every strain one should look for the medium which

Table 3. *The effect of F state on the broth culture stability of strains of Escherichia coli K 12 with different phenotypes*

The datum is the log-transformation of a nephelometrical reading.

Strain no.	Mating type	Phenotype	Infusion broth	Nutrient
			I.S.M.	broth NB5
			log nephelometer reading	
256 _B	F-	Lac- Mal- Gal- Xyl- Ara- Mtl- St ^r	1.734 ± 0.009	2.030 ± 0.029
	F+		1.609 ± 0.019	1.522 ± 0.043
176	F-	B- M- T ₁ ^r	2.059 ± 0.022	2.528 ± 0.020
243	F+		1.630 ± 0.006	1.728 ± 0.132
180	F-	B- M- T ₁ ^r St ^r	2.581 ± 0.014	2.036 ± 0.023
244	F+		1.570 ± 0.024	1.653 ± 0.010
42	F-	T- L- Th- Lac- Mal- Gal- Ara- Xyl- Mtl-	2.180 ± 0.036	2.423 ± 0.024
230	F+		1.633 ± 0.014	1.464 ± 0.025
129	F-	T- L- Th- Lac- Mal- Gal- Ara- Xyl- Mtl- St ^r	1.787 ± 0.022	2.069 ± 0.026
184	F+		1.460 ± 0.018	1.595 ± 0.024
361	F-	C- Gal _A - T ₁ ^r T ₆ ^r St ^r Lp ^s	2.680 ± 0.023	2.522 ± 0.074
	F+		2.308 ± 0.001	2.485 ± 0.041
363 _A	F-	C- Gal _A - T ₁ ^r T ₆ ^r St ^r Lp ⁺	2.622 ± 0.044	2.527 ± 0.011
	F+		2.103 ± 0.016	2.457 ± 0.289
365 _C	F-	C- H- Gal _A - T ₁ ^r Lp ^s	2.645 ± 0.042	2.268 ± 0.165
	F+		1.723 ± 0.019	1.766 ± 0.076
324 ⁽⁺⁾	F-	H-	2.694 ± 0.019	2.442 ± 0.015
	F+		2.291 ± 0.027	2.352 ± 0.053
367	F-	H- Lac- Ara-	2.808 ± 0.039	2.742 ± 0.030
	F+		2.107 ± 0.030	2.403 ± 0.043
354	F-	H- T ₆ ^r Val ^r	2.903 ± 0.036	2.735 ± 0.025
	F+		2.024 ± 0.019	2.104 ± 0.010
368	F-	H- Mal- Gal _A - T ₁ ^r Val ^r Az ^s Lp ^s	2.652 ± 0.039	2.223 ± 0.011
	F+		2.275 ± 0.033	2.151 ± 0.135
369	F-	H- Mal- Gal _A - T ₁ ^r Val ^r Az ^r Lp ⁺	2.754 ± 0.016	2.578 ± 0.017
	F+		2.027 ± 0.014	1.676 ± 0.029
337	F-	Pr- T ₁ ^r Az ^r	2.870 ± 0.009	2.473 ± 0.018
	F+		2.175 ± 0.033	2.158 ± 0.052
349	F-	Pr- T ₁ ^r T ₆ ^r Val ^r Az ^r	2.890 ± 0.031	2.688 ± 0.035
	F+		2.295 ± 0.010	2.238 ± 0.018
364	F-	Pr- Gal- T ₁ ^r T ₆ ^r Val ^r Az ^r	2.745 ± 0.010	2.364 ± 0.073
	F+		2.205 ± 0.027	2.183 ± 0.024
371	F-	Pr- Gal- Ara- T ₁ ^r T ₆ ^r Val ^r Az ^r	2.951 ± 0.034	2.865 ± 0.032
	F+		2.474 ± 0.016	2.189 ± 0.031

(+) This strain has given inconsistent results in subsequent experiments.

Figures reported are means (± standard errors) of four subcultures.

Meaning of symbols:

Biochemical deficiencies: B=biotin; M=methionine; T=threonine; L=leucine; Th=thiamine; C=cystine; H=histidine; P=proline.

Sugar fermentations: Lac=lactose; Mal=maltose; Gal=galactose; Xyl=xylose; Ara=arabinose; Mtl=mannitol.

Virus resistances: T₁=bacteriophage T₁; T₆=bacteriophage T₆.

Drug resistances: Az=sodium azide; St=streptomycin; Val=valine.

Lysogenicity: Lp^s=sensitive to phage λ; Lp⁺=lysogenic for phage λ.

yields the maximum difference in broth culture stability between the F- and the F+ variants of the same phenotype. Accordingly, a statistical analysis of data reported in Table 3, besides showing the impressive effect of F ($P < 0.001$) reveals a highly significant effect of the medium. Moreover (Table 4), the latter is significantly interacting with the effect of F which is, on the whole, appreciably greater in the infusion broth I.S.M. than in nutrient broth NB5. Also the interaction 'F $\times$ phenotype' is significant.

Table 4. *General means ($\pm$ standard error) of data collected in Table 3*

	F-	F+	
Nutrient broth NB5	2.442 ± 0.032	2.007 ± 0.045	2.225 ± 0.034
Infusion broth I.S.M.	2.562 ± 0.046	1.995 ± 0.038	2.279 ± 0.038
	2.502 ± 0.028	2.001 ± 0.029	

The two main effects: 'F' and 'medium' and their interaction are highly significant ($P < 0.001$).

The 8 strains on which the acid-agglutination point was determined were submitted to a more extensive study of their cultural pattern: 40 clones of each strain were examined nephelometrically (Fig. 2).

The mean values of the log-transformation of nephelometrical readings are: 2.070 ± 0.008 for the M_1 strains versus 1.918 ± 0.009 for the M_2 strains; 2.030 ± 0.009 for the St^+ strains versus 1.958 ± 0.007 for the St^- ones; 2.247 ± 0.010 for the F- strains versus 1.740 ± 0.007 for the F+ ones. All these differences are highly significant ($P < 0.01$). The interaction 'markers $\times$ streptomycin resistance' is irrelevant; the interaction 'streptomycin resistance $\times$ F' is highly significant ($P < 0.01$) in the sense that the effect of F is greater in St^+ strains.

F+ and F- in rough and smooth forms

It has long been known that broth cultures of bacteria in the rough form are very much more unstable than smooth cultures. Therefore, the question arises as to how much of the difference in culture stability between F+ and F- strains is due to a morphological difference. In fact, F- strains, when plated on nutrient agar, produce a majority of smooth colonies (about 70-90 %) which is decreased to a minority (about 20-40 %) in F+ strains.

Moreover, there is the same shift in the distribution of smooth colonies during the course of infection of an F- strain by an F+ one. But data of this type are inadequate to give a quantitative knowledge of the impact of rough/smooth dissociation on the cultural pattern of compatible and incompatible strains. Therefore a number of subcultures from rough and smooth colonies (care was taken to discard colonies of intermediate morphology) were studied nephelometrically. The investigation was made on all 8 strains quoted above with an experimental design enabling factorialization of: (1) F state; (2) rough/smooth form; (3) streptomycin resistance. Part of the results of these experiments are shown in Fig. 3. Averages calculated on the full set of experimental results are reported in Table 5. A statistical analysis of these data shows that,

Fig. 3. The impact of S-R dissociation on the broth culture stability of F+ and F- strains. Medium: nutrient broth NB5.

Table 5. The impact of F state (F-|F+), morphological form (rough (R) or smooth (S)) streptomycin resistance (St^s|St^r) on the stability of broth cultures of *Escherichia coli* K12.

The datum is the log-transformation of a nephelometric reading. Figures reported are means and standard errors of 80 subcultures of which 40 are from a TLB₁- strain and 40 from a BM-. The medium was nutrient broth NB5.

	F-	F+	
S {	St ^s	2.761 ± 0.011	2.121 ± 0.028
	St ^r	2.310 ± 0.044	
R {	St ^s	2.287 ± 0.021	1.883 ± 0.020
	St ^r	2.053 ± 0.036	
		2.353 ± 0.021	1.651 ± 0.008

The mean value for St^s is 2.082 ± 0.027; that for St^r is 1.922 ± 0.022.

besides the main effects just mentioned, namely, (1) mating type; (2) rough/smooth form; (3) streptomycin resistance (F, Ph and St), the following interactions of first and second order are also highly significant ($P < 0.001$): (1) the effect of F is greater in smooth, i.e. the effect of Ph is greater in F +; this interaction is greater in St⁺; (2) the effect of F is greater in St⁺, i.e. the effect of St is greater in F —, this interaction is greater in smooth; (3) the effect of Ph is greater in St⁺, i.e. the effect of St is greater in smooth; this interaction is greater in F —. On the whole it is clear that broth culture stability is affected by F state and rough/smooth state quite independently.

The cultural behaviour as a co-ordinate for the time-course of infection

Provided the difference in F state is highly correlated with the stability of broth cultures and that morphological dissociation cannot account for such correlation, it seemed legitimate to attempt a description of the kinetics of F infection in terms of cultural behaviour. With this aim, strain 176 (F — Lac +) has been infected with strain 230 (F + Lac —) under experimental conditions already described. The mixture of the two strains was plated at intervals on EMB, whence 40 colonies were transferred to infusion broth I.S.M. for a test of cultural stability. Results are shown in Fig. 4.

Analogous results have been obtained with infection of strain 176 by strain 184, of strain 42 by strains 243 and 244, of strain 129 by strains 243 and 244, of strain 180 by strains 230 and 184.

When colonies of the donor strain were isolated at intervals during the infection and used for a test of cultural stability no modifications of this property were detected in them.

Particular attention was given to the effect of changes in experimental conditions on the kinetics of the transformation of cultural behaviour, with the aim of establishing what degree of standardization would be needed in further researches. It was found that this transformation is not appreciably affected by variations of temperature between 35 and 39° nor by variations of pH between 6.4 and 7.5. On the contrary it is strongly enhanced by agitation of the mixture of organisms. Transformation of the cultural behaviour was not obtained when infection was attempted in distilled water, saline and minimal medium unless these media were supplemented with yeast extract (0.5%). The effect of the initial ratio of the number of F — organisms to that of F + was tested by simultaneous infections of strain 176 with strain 230 at different F — to F + ratios ranging from 1:8 to 8:1 on a twofold scale. Results are given in Fig. 5 and clearly show the importance of the relative concentration of the donor and the acceptor strains. In fact, when 40 clones of strain 42 and strain 176, infected respectively with strains 243 and 230, were screened as F + and F — on the basis of their cultural behaviour and then submitted to recombination tests, results were fully consistent.

DISCUSSION

The main result of this investigation is the detection of properties other than compatibility which are correlated with the F+ state, at least as far as our strains of *Escherichia coli* are concerned. These properties, namely: cultural

Fig. 4. The time-course of the infective transformation of broth culture stability. Strain 176 (F-) has been infected by strain 280 (F+) to obtain strain 243 (F+). Medium: infusion broth I.S.M.

behaviour, acid agglutination and affinity for stains, are open to a unitary interpretation in terms of the surface properties of the bacteria. In fact, all experimental results indicate very coherently that the F- organisms behave as if they were more negatively charged than F+.

Of course, whether homogeneous dispersions or agglutination occur, either spontaneously or because of a critical potential, must depend upon the balance of the cohesive and repulsive forces which are related to many factors, like hydration of ionizing and non-ionizing polar groups, adsorption of ions at the surface of the organism, proper electric charges of the organisms, etc. (Lamanna & Mallette, 1953). Therefore, at this stage of our knowledge, to speak in terms of surface charges has no other validity than that of a simplification making easier the shaping of a model, which could be stated as follows.

Between bacteria suspended in a medium, cohesive and repulsive forces are produced, according to their surface charges. Sex compatibility of two strains is influenced by this interaction which is defined by the relative no less than by the absolute values of the charges. Self-compatible strains differ from the others in a surface structure which can be induced by contact (infection).

Fig. 5. Effect of varying the ratio of F^+ to F^- cells on the efficiency of transformation of broth culture stability. Small circles each represent means ($\pm$ standard error) of forty data. Medium: nutrient broth NB5.

All experimental results here reported are in agreement with this model but, unless supporting proofs of a more direct nature can be offered, the mere observation of a correlation of sex compatibility and surface properties is no reason to accept a cause-and-effect relationship. On the other hand, some supporting data are not to be dismissed:

(1) Various F^+ strains have different F^+ strengths and the relative behaviour of the two strains depends on the difference of F^+ strengths between the two (Cavalli *et al.* 1953).

(2) BM^- strains which have a mean acid agglutination point significantly lower than that of TLB_1^- strains are weaker F^+ than the latter (Table 2 of this paper; and Cavalli *et al.* 1953).

(3) Electrophoretic mobility drops to a minimum (Abramson, Moyer & Gorin, 1942) and frequency of recombination rises to a maximum (Maccacaro, 1953) during the early phases of growth of a culture.

(4) Oxygenation of a BM- F+ culture converts it into a reversible F-phenocopy (Lederberg *et al.* 1952). Exposure of cultures of *Staphylococcus pyogenes* to oxygen prevents the effect of agents which would make the organisms less electronegative (Lerche, 1953).

(5) No morphological equivalents of sex compatibility have been detected at the cell surface by electron microscopy (Maccacaro & Pasinetti, 1955).

Needless to say, many other facts cannot be fitted at this stage, within the model sketched above. But in whatever way the available evidence is taken, to say that compatibility and surface properties are highly correlated seems a safe conclusion, resting on the following points which seem proved beyond any doubt:

(1) Between the F- and the F+ variants of every tested strain there is a uniform difference in surface properties.

(2) Compatibility and surface properties are transmitted infectively from F+ to F- strains along the same time-course.

(3) Screening of infected strains for cultural behaviour yields results fully consistent with scoring for compatibility.

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