The Compatibility of Hly Factor, a Transmissible Element which Controls α -Haemolysin Production in *Escherichia coli*

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The transmissible nature of the genetic factor in *Escherichia coli*, which controls α -haemolysin production, was recognized by Smith & Halls (1967). The element responsible was termed Hly. The fi^+ character of Hly factor was described at the same time.

Goebel & Schrempf (1971) demonstrated the presence in the cytoplasm of a haemolytic *E. coli* strain of a covalently-closed circular DNA molecule with a contour length of $29.5 \pm 0.5 \mu m$, which was transferred to non-haemolytic strains together with the haemolytic character. Therefore this DNA species represents the Hly factor. However, Hly factor was not taken into account by Hedges & Datta (1972), who classified the F-like plasmids into four compatibility groups. A further group of F-like plasmids was subsequently added by Naomi Datta in 1974 (personal communication), *Folac* being the prototype of it (Falkow & Baron, 1962).

In the present paper we show that Hly factor determines the production of F-type pili and that it is compatible with F-like plasmids of all the five groups previously described.

Escherichia coli strain P212 carrying Hly factor was received from H. Williams Smith. *Escherichia coli* strains J53 and C600, carrying many known plasmids, were provided by Naomi Datta. R7, R89 and R120, three F-like R factors recently identified in Trieste, were also used.

Crosses were carried out by mixing equal numbers (about $2 \times 10^7/ml$) of donor and recipient cells in nutrient broth, which was subsequently incubated overnight at 37 °C. Colonies were then counted on nutrient or blood agar containing, if required, appropriated amounts of different antibiotics to define the number of donor, recipient and recombinant cells. Colonies producing colicin were identified by standard techniques. To test for the compatibility of plasmids, recombinant clones were purified on nonselective medium and tested by replica plating for the presence of each plasmid.

The fi^+ character of Hly factor was confirmed in our study. Moreover, we could show that *E. coli* strains carrying the Hly factor allowed a small but definite increase in the titre of MS2 phage (Davis, Strauss & Sinsheimer, 1961). This demonstrates that Hly controls the synthesis of F-pili.

Being not perfectly clear from the data reported by Smith & Linggood (1970) and Smith & Heller (1973) whether Hly is self-transmissible or mobilized by the F factor, we tested different strains of *E. coli* K12 F⁻, which had acquired the Hly factor from *E. coli* strain P212, for sensitivity to the female-specific phages T7 and $\phi 2$ (Makela, Makela & Soikkeli, 1964; Cuzin, 1965). The Hly-positive bacteria remained sensitive to these phages. Hly factor was transmissible from these bacteria by conjugation. It appears, therefore, that at least the Hly factor used in the present study, for which we propose the name of Hly-P212, is self-transmissible.

Table 1. *Compatibility of Hly-P212 factor*

Plasmid in the donor	Plasmid in the recipient	Compatibility group of plasmid*	Frequency of transfer/recipient cell	Analysis of recombinants† (Number of colonies tested)		
				Total	With Hly-P212	With the other plasmid
Hly-P212	—	—	4×10^{-1}	—	—	—
	<i>Flac</i>	FI	1×10^{-1}	136	136	136
	R386	FI	1×10^{-1}	92	92	92
	R136	FII	1×10^{-1}	102	102	102
	ColB-K98	FIII	$< 10^{-6}$	No recombinant formed		
	R124	FIV	4×10^{-2}	28	28	28
	<i>Folac</i>	FV	2×10^{-2}	154	154	154
<i>Flac</i>	—	FI	1×10^{-1}	—	—	—
	Hly-P212	FI	1×10^{-1}	163	163	163
R386	—	FI	4×10^{-1}	—	—	—
	Hly-P212	FI	8×10^{-5}	24	24	24
R136	—	FII	1×10^{-1}	—	—	—
	Hly-P212	FII	2×10^{-3}	63	63	63
ColB-K98	—	FIII	2×10^{-1}	—	—	—
	Hly-P212	FIII	2×10^{-2}	188	188	188
R124	—	FIV	1×10^{-2}	—	—	—
	Hly-P212	FIV	2×10^{-7}	17	17	17
<i>Folac</i>	—	FV	1×10^{-1}	—	—	—
	Hly-P212	FV	1×10^{-3}	226	226	226

* Other than Hly-P212.

† Derived from 3 to 8 purified clones for each cross.

Hly-P212 factor does not control resistance to any known antibiotic or production of any known colicin, since cells, which acquire the haemolytic character in crosses between Hly⁺ and Hly⁻ strains, do not modify their antibiotic sensitivity pattern or become producers of colicin.

Crosses between strains carrying the Hly-P212 factor and plasmids representative of groups FI, FII, FIII, FIV and FV always yielded stable doubles (Table 1). Stable doubles were also formed between Hly-P212 and three recently identified F-like R factors, whose compatibility behaviour will be reported elsewhere. Finally, Hly-P212 was found to be compatible with R factors of groups A, C, I α , I β , I ω , N, P, T and W (Hedges, 1974). In each case, Hly-P212 and the other factor coexisted in doubles as separate entities, as shown by their independent transmission in conjugation tests.

Our data support the conclusion of Hedges & Datta (1973) that there is no strict correlation between compatibility and surface exclusion. In fact, Hly-P212 is compatible with all the F-like plasmids we tested, but in some crosses exclusion was very efficient, mainly when R386 or R124 was introduced into a strain carrying Hly-P212. ColB-K98 excluded Hly-P212 so efficiently that no recombinant was obtained in this cross. No case of exclusion was recorded in crosses between Hly-P212 and the plasmids of the non-F-like groups.

Our results demonstrate that the Hly-P212 factor, which controls the production of F-type pili, is compatible with all the F-like plasmids tested; it can therefore be considered the first member of a new compatibility group, which according to Hedges & Datta (1972) should be called FVI.

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