INHIBITION OF BACTERIAL CONJUGATION BY CELL WALL PREPARATIONS[1]

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The restrictions imposed by the specificity of the association of competent mating cells must, in some measure, contribute to species identity. Such restrictions would be operative in an environment containing different microbial species capable of sexual reproduction. During sexual reproduction in bacteria, it is reasonable to assume that the initial effective surface contact between male and female cells involves areas of complementary structure (Sneath and Lederberg, 1961) and that this initial linkage is followed by the formation of a conjugation bridge. If this assumption is correct it would be expected that the cell wall derived from the male or female cell would inhibit conjugation between viable male and female cells by competition for complementary sites. In this report the inhibition of conjugation in E. coli by cell wall will be described.

Materials and Methods

The bacterial strains[2] used are: E. coli C600B (Female F^-, Thr^-, Leu^-, B_1^- streptomycin resistant), E. coli 1895 (Male HFrC, $Meth^-$, strep-

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2) E. coli, strains C600B, 1895 and B were generously furnished by Dr. Dale Kaiser, Department of Biochemistry, Stanford University School of Medicine. Alcaligenes faecalis was kindly provided by Dr. Alex Sonnenwirth of Jewish Hospital, St. Louis.

tomycin sensitive), E. coli B, and Alcaligenes faecalis. Cells were grown on Difco Antibiotic Medium 3. In conjugation studies about 10^8 cells each of C600B and 1895 with additions as described in the tables were mixed in an 18 x 150 tube in a total volume of 1.0 ml of half strength Antibiotic Medium 3. After incubation for 1 hr. at 37° the diluted cultures were spread on agar plates containing minimal salts medium enriched with glucose (0.8%), thiamine (10 γ/ml) and streptomycin (100 γ/ml). Colony counts were made at 48 hours.

Bacterial cell wall was isolated by differential centrifugation after grinding cells with alumina in a mortar. The wall preparations from E. coli were identical in absorption spectrum with that previously described (Salton and Horne, 1951). Cell membrane preparations were made from protoplasts (Chargaff et al., 1957) lysed in 10^{-3} M Mg^{++} and isolated by centrifugation (Abrams and McNamara, 1962).

Results and Discussion

As shown in Table I, as little as 0.2 mg of male or female cell wall markedly inhibits the number of recombinants resulting from the mating of

Table I
Effect of Cell Wall on Conjugation+

Cell Wall Source	Cell Wall Dry Wt.	Recombinants per 10^{-3} ml.	Cell Wall Source	Cell Wall Dry Wt.	Recombinants per 10^{-3} ml.
	mg.			mg.	
Control	-	1760	Control	-	1760
C600B	5.0	34	1895	5.0	30
"	1.0	78	"	1.0	56
"	0.2	253	"	0.2	161
"	0.04	720	"	0.04	920

+ See Materials and Methods.

C600B with 1895. The effect of 0.04 mg of cell wall is questionable because of the inaccuracies inherent in an assay of this type.

The specificity of the inhibition of conjugation is described in Table II. Cell wall from the gram negative rod Alcaligenes faecalis was virtually non-inhibitory[3] while E. coli B cell wall was as inhibitory as cell wall from C600B or 1895. The weak inhibitory effect observed with C600B cell membrane is probably caused by cell wall components contaminating the membrane preparation.

Table II
Specificity of Inhibition of Conjugation +

Expt. #	Cell Wall or Membrane Source	Dry Wt.	Recombinants per 10^{-3} ml.
		mg.	
1	Control	-	1460
	A. faecalis (wall)	0.2	870
	"	1.0	920
	E. coli B (wall)	0.2	134
	"	1.0	49
	E. coli 1895 (wall)	0.2	249
	"	1.0	100
2	Control	-	1500
	E. coli (C600B) (wall)	0.2	490
	"	1.0	210
	E. coli (C600B) (memb.)*	0.2	950
	"	1.0	550
	"	5.0	433

* Cell membrane (see text).
\+ See Materials and methods.

3) Essentially the same result was obtained in three other experiments even with concentrations as high as 5.0 mg of cell wall.

The effect of time of addition of cell wall on conjugation is given in Table III. These data show that maximal inhibition is attained during early stages of conjugation. Moreover the lack of inhibition by the delayed addition of cell wall (30 mins.) indicates that the observed inhibition (0 mins.) is not due to a loss in cell viability resulting from the action of any colicine-like substance (Goebel, 1962).

Table III
Effect of Time of Addition of Cell Wall on Conjugation +

Cell Wall Added			Recombinants	Cell Wall Added			Recombinants
Source	Amt.	Time	per 10^{-3} ml.	Source	Amt.	Time	per 10^{-3} ml.
	mg.	min.			mg.	min.	
Control	-	-	990	Control	-	-	1480
C600B	1.0	0	145	1895	1.0	0	60
"	"	5	390	"	"	5	257
"	"	10	740	"	"	10	490
"	"	20	1060	"	"	20	1220
"	"	30	940	"	"	30	1250

+ See Materials and Methods.

The data shown in the tables are consistent with the view that the cell wall exerts its effect during conjugation by competition for complementary sites involved in the initial contact of mating cells.

It has been reported that the "conjugal substance" of viable male and female cells exhibits a differential lability to periodate treatment (Sneath and Lederberg, 1961). In addition, these investigators observed that periodate treated male cells can act as feeble females. Attempts, in our studies, to differentiate male and female cell wall by treatment with periodate, heat, acid, alkali, and detergent have thus far been unsuccessful. The failure to observe a differential lability of male and female cell wall could be due to

the difference in bacterial strains. Moreover, if the male cell wall preparation contains in addition to male components, a sufficient quantity of female components, a differential lability would not be detected by this assay.

A better understanding of this phenomenon requires the purification and characterization of the inhibitory components associated with cell wall. Recently, we have been able to solubilize the inhibitory components from both male and female cell wall. The solubilization procedures and a report on the nature of the purified components will be published in detail elsewhere.

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