

Attachment of *Bdellovibrio bacteriovorus* to Cell Wall Mutants of *Salmonella* spp. and *Escherichia coli*

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Bdellovibrio bacteriovorus is a parasitic bacterium which attaches to the cell walls of other kinds of bacteria, penetrates the wall and multiplies in the space between the wall and the cell membrane (6, 8). We studied the effect of the several strains of *Salmonella* spp. and *Escherichia coli*. The cell wall composition of the bacterial mutants used as hosts is described in Table 1. Bacteria were grown and attachment experiments were carried out as previously described (9). A

TABLE 1. Attachment of *B. bacteriovorus* 109 to smooth and rough *Salmonella* spp. and *Escherichia coli*^a

Colony type	Chemotype	Cell wall composition	<i>S. typhimurium</i>			<i>S. minnesota</i>			<i>E. coli</i>		
			Strain	Attachment of 50% <i>Bdellovibrio</i> population (min)	<i>Bdellovibrio</i> attached at 20 min (per cent of total <i>Bdellovibrio</i>)	Strain	Attachment of 50% <i>Bdellovibrio</i> population (min)	<i>Bdellovibrio</i> attached at 20 min (per cent of total <i>Bdellovibrio</i>)	Strain	Attachment of 50% <i>Bdellovibrio</i> population (min)	<i>Bdellovibrio</i> attached at 20 min (per cent of total <i>Bdellovibrio</i>)
Smooth		Rough core and O-specific side chains (complete O antigen)	LT2	> 20.0	39.3	S99	> 20.0	38.5	O111	> 20.0	9.4
Rough	Ra	Rough core (complete R antigen)	TV-119	4.4	75.8	R60	7.4	80.0			
	Rb	Rough-core polysaccharide, lacks glucosamine	TV-161	7.5	64.0	R345	14.4	53.5			
	Rc	Rough-core polysaccharide, lacks glucosamine and galactose							J5	18.3	53.2
	Re	Rough-core polysaccharide, lacks all hexoses and heptoses	G30/C21	10.5	56.7						

^a The classification of the host bacteria is based on the principles proposed by O. Lüderitz et al. (3) and the studies of E. C. Heath et al. (1), O. Lüderitz et al. (2), N. Nikaido et al. (4), and M. J. Osborn et al. (5).

chemical structure of the host cell wall on the first step (the attachment process) in the interaction of *Bdellovibrio* and its host, and our results are reported in this note.

The kinetics of attachment of *Bdellovibrio* strains 109 (ATCC 15143) and GB (isolated in our laboratory from sewage) were followed on

slight modification of the original growth technique was introduced here: 10^8 to 2×10^8 plaque-forming units of *Bdellovibrio* and 10^9 to 2×10^9 *E. coli* B (ATCC 15144) cells were simultaneously inoculated into 15 ml of dilute NB medium (7) and were incubated for 16 to 20 hr.

Two parameters were chosen for measuring

the kinetics of attachment: (i) the time required for attachment of 50% of the total *Bdellovibrio* population, and (ii) the per cent of *Bdellovibrio* cells attached (i.e., the percentage of the total number) within 20 min of incubation. The 20-min period was chosen since the attachment rate usually slows down by this time and approaches a plateau.

Table 1 and Fig. 1 summarize the ability of the host mutants to adsorb *Bdellovibrio* strains 109 and GB. For both *Bdellovibrio* strains, the host bacteria lacking the O-specific side chains but containing a complete "rough" core (chemotype Ra) were better receptors than their wild-type (smooth) strains. This is indicated by a more rapid rate of attachment and a higher per cent of attached cells at 20 min. The low attachment to smooth bacteria could not be caused by a lack of host cells, since they were provided in great excess. Double and triple concentrations of the host cells yielded the same kinetics and plateau.

The absence of glucosamine from the basal structure of rough host mutants (chemotype Rb) significantly reduced the attachment and plateau level compared to that of the mutant having the complete rough core. Additional deficiencies in

the R antigen (chemotype Re) further reduced receptor activity, pointing to the possible location of receptors for *Bdellovibrio* in the R antigen.

The addition of rough host bacteria to the mixture of *Bdellovibrio* 109 and *S. typhimurium* LT2 resulted in an immediate increase in the number of *Bdellovibrio* cells attached and led to the plateau usually obtained with the rough strain (Fig. 2). Essentially, the same picture was obtained when *E. coli* J5 was added to a mixture of *Bdellovibrio* 109 and *E. coli* O111. This suggests that the *Bdellovibrio* population may be heterogeneous, consisting of (i) cells capable of efficient attachment to both rough and smooth strains and (ii) cells capable of efficient attachment only to rough strains. Perhaps the difference between these two groups is that only the former is able to pass through the barrier of the O side chains to the inner R antigen within 20 min.

Within the experimental error of the method used (9), the efficiency of plating (relative to that on strain LT2) was the same when each of the two *Bdellovibrio* strains was plated on any of the four strains of *S. typhimurium* or the two strains of *E. coli*. Thus, differences in the attachment efficiency do not necessarily lead to differences in the efficiency of plating. Since the plaque assay is completed in 4 to 6 days, there is sufficient time for all the *Bdellovibrio* cells to attach. It should be mentioned that *Bdellovibrio*, in contrast to bacteriophage, is capable of attacking stationary-phase bacteria.

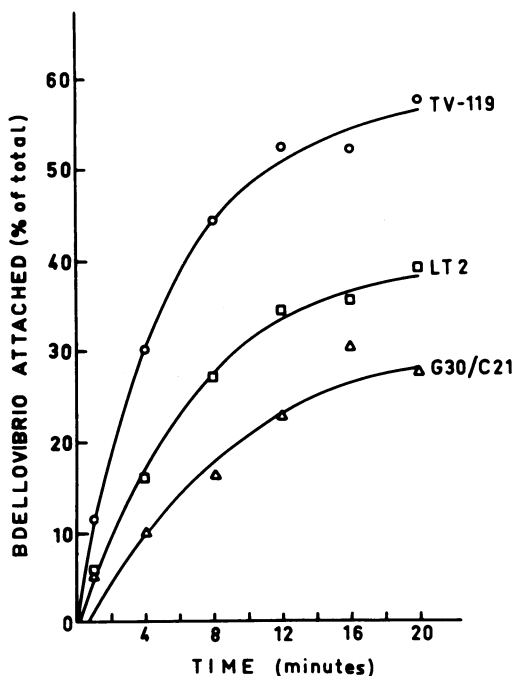


FIG. 1. Attachment of *B. bacteriovorus* GB to smooth (LT2) and rough (TV-119 and G-30/C21) *S. typhimurium*.

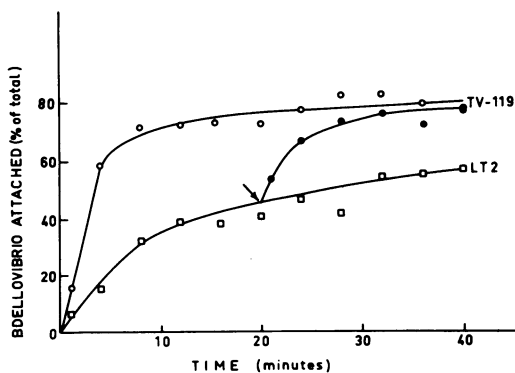


FIG. 2. Attachment of *B. bacteriovorus* 109 to smooth (LT2) and rough (TV-119) *S. typhimurium*. Initially, *Bdellovibrio* (1.5×10^8 cell/ml) and host (1.5×10^8 cells/ml) cells were mixed and incubated at 30 C with shaking. After 20 min, a concentrated suspension ($10 \times$ the initial host concentration) of TV-119 was added (arrow) to the incubation mixture containing LT2 as host and was incubated for an additional 20 min.

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