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Isolation of Cell Surface Antigen Mutants of *Myxococcus xanthus* by Use of Monoclonal Antibodies

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Monoclonal antibodies (MAbs) with affinities for molecules on the cell surface of the procaryote *Myxococcus xanthus* were used in a screening strategy for the isolation of mutants lacking particular cell surface molecules. From a large library of independent mutants created by Tn*5* transposon mutagenesis, mutants were isolated which lacked reactivities with MAb 1604 (a MAb specific for a cell surface protein) and MAbs 2600, 1733, 1514, 1412, and 783 (MAbs specific for carbohydrate epitopes on the O antigen of lipopolysaccharide [LPS]). The defect in antibody recognition was shown by genetic crosses and DNA hybridization experiments to be caused by the Tn*5* transposon acting as a mutation at a single locus. Quantitative enzyme-linked immunosorbent assays showed that particular mutant strains had no detectable affinity for the specific MAb probe. LPS mutants were resistant to myxophage Mx8, and this provided a selection method for isolating a large number of new LPS mutants. A class of Mx8-resistant mutants lacked reactivity with MAb 1514 and therefore was defective in the O antigen of LPS. A class of Mx1-resistant mutants lacked reactivity with MAb 2254, a MAb specific for a carbohydrate epitope on the core of LPS. A comparison of MAb binding to different mutant strains revealed a principle for mapping epitopes and showed that MAbs 1514 and 2254 recognize side-chain carbohydrates rather than backbone carbohydrates within the LPS molecule.

Molecules on the cell surface of *Myxococcus xanthus* must mediate the cell-cell interactions which are important to the social behavior of the organism (18). Very little is known about these cell surface molecules or their functions, particularly functions crucial to the complex developmental cycle which culminates in the production of multicellular structures called fruiting bodies.

Monoclonal antibodies (MAbs) which recognize the cell surface of *M. xanthus* have been isolated and used as probes for identifying molecules possibly important during development (8–10). These MAbs also have been used as tools for purifying and characterizing these cell surface molecules (10). Potentially, these MAbs could be used for isolating antigen-minus mutants. This approach would not require prior knowledge about the function of a particular cell surface molecule and could lead to the genetic analysis of molecules important to the social behavior of *M. xanthus*.

We have shown that six MAbs (2600, 1733, 1514, 1412, 783, and 2254) recognize carbohydrate epitopes on lipopolysaccharide (LPS) isolated from *M. xanthus* (6). We have used these MAbs to show that the LPS consists of a heterogeneous population of molecules containing the three classical covalently attached regions of LPS: lipid A, which is embedded in the outer membrane; core, which contains a chain of carbohydrates; and O antigen, which contains a variable number of repeating oligosaccharide units. The variability in the number of O-antigen units results in a population of molecules migrating in a stepladder pattern during electrophoresis. Small modifications in some of the LPS molecules can result in a stepladder containing doublet bands. We have shown that four of the MAbs (2600, 1733, 1514, and 1412) recognize a stepladder of doublet bands corresponding to O-antigen epitopes. One MAb (783) recognizes a stepladder of singlet bands, indicating that it may recognize an O-antigen epitope which can be modified in some of the LPS molecules. This type of modification of a cell surface molecule could be important in cell-cell interactions (16). We have shown also that one MAb (2254) recognizes the core region of the LPS.

MAb 1604, a MAb against a cell surface protein, has been shown to recognize three protein bands (200, 170, and 140 kilodaltons) on a Western immunoblot (9). This MAb has been used to immuno-affinity purify the corresponding cell surface antigen (csa 1604) (10).

In this study, we used the MAbs specific for the cell surface of *M. xanthus* to isolate mutants which are lacking the corresponding epitopes. We isolated a mutant which is missing the MAb 1604 epitope as well as mutants which are missing LPS epitopes. We showed that the LPS mutants are resistant to *M. xanthus* bacteriophage, and we have used bacteriophage resistance to select for additional mutants defective in the biosynthesis of LPS.

MATERIALS AND METHODS

Bacteria and bacteriophages. *M. xanthus* strains were grown in CT (5) or CTT (2) liquid at 32°C on a gyratory shaker. Growth on solid medium was on CTT plates (12) at 32°C. Kanamycin-resistant cells were selected for, and maintained, in the presence of 70 μg of kanamycin monosulfate per ml. Yellow swarming cells were used whenever possible.

Escherichia coli strains were grown in LB (13) liquid with vigorous shaking at 37°C. Growth on solid medium was on LB plates at 37°C.

Myxococcus bacteriophage plate lysates were prepared as previously described (15). Myxophages Mx1 (3), Mx8riv1 (15), and Mx9 (14) were grown at 32°C. Mx4 *hft hrm*(Ts) (4) was grown at 28°C.

Bacteriophage sensitivity was assayed by streaking the bacteriophage (greater than 5×10^8 PFU/ml) on a lawn of bacteria in soft agar.

To assay for adsorption, 1 ml of cells (4×10^8 cells per ml) was mixed with 0.1 ml of bacteriophage (4×10^8 PFU/ml) and was allowed to adsorb for 20 min at room temperature. Four milliliters of CT was added, and the cells were pelleted

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at 12,100 × *g* for 5 min. The supernatant was assayed for remaining bacteriophage by spotting 3-μl samples of twofold dilutions on a lawn of indicator bacteria (20).

E. coli bacteriophage P1::Tn*5* lysates were made on *E. coli* C600 as previously described (12).

M. xanthus was transduced with P1::Tn*5* as previously described (12).

M. xanthus was transduced with Mx4 *hft hrm*(Ts) (4) by adding 0.5 ml of cells (5×10^8 cells per ml) to 0.1 ml of transducing lysate (multiplicity of infection, 0.1 to 1.0) and incubating at 32°C for 20 min. The transducing mix was then plated out in 2.5 ml of CTT top agar on CTT plates containing 70 μg of kanamycin monosulfate per ml; the plates were incubated at 32°C for 4 to 6 days.

Construction and screening of the Tn*5* mutant library. Each JZ007 P1::Tn*5* transductant was toothpicked into a separate well of a 96-well tissue culture plate (Corning Glass Works); each well contained 200 μl of CT containing kanamycin monosulfate at 70 μg/ml. The 96-well plates were slowly shaken at 32°C in a moist incubator until the cells reached mid-log phase (approximately 2 days). Glycerol was then added to 20%, and the cells were frozen in the 96-well plates at −70°C.

Cells were transferred from frozen 96-well plates by scraping the tops of the wells with octapette tips and inoculating the scraped cells into new 96-well plates containing 200 μl of CT (70 μg of kanamycin sulfate per ml) per well. The 96-well plates were slowly shaken at 32°C until the cells reached mid-log phase (approximately 2 days). Nitrocellulose was cut into rectangles (3 by 4.25 in. [1 in. = 2.54 cm]), was wetted, and was placed over the holes of an empty modified 96-tip Costar rack (the rack was modified by drilling a hole in its side so that it could be connected with vacuum tubing to the house vacuum). The vacuum was turned on, thus sucking the nitrocellulose sheet onto the top of the rack and making small concave indents above the 96 holes in the Costar rack. Samples (25 μl) of log-phase cells were transferred from each well of the 96-well plate onto each corresponding concave spot on the nitrocellulose. The nitrocellulose blots were then screened by the following procedure (all of the subsequent steps were done at room temperature at a slow setting on a platform shaker): a 10-min wash in TBST (20 mM Tris hydrochloride [pH 7.5]–150 mM NaCl, and 0.05% Tween 20), two 30-min incubations with TBST containing 1.5% gelatin (wt/vol), three 10-min washes in TBST, incubation with a 1/50 to 1/400 dilution (depending on the MAb used) of MAb-containing ascites fluid in TBST for 1 h, three 10-min washes in TBST, incubation with a 1/750 dilution of alkaline phosphatase-conjugated goat anti-mouse immunoglobulins (Cooper) in TBST for 1 h, two 10-min washes in TBST, two 10-min washes in TBS, two 10-min washes in Veronal-acetate buffer (150 mM sodium barbital adjusted to pH 9.6 with glacial acetic acid), and a 10- to 30-min incubation in the substrate solution (1) (150 mM sodium barbital [pH 9.6]–4 mM $MgSO_4$–0.115 mM BCIP [dissolved in a minimal volume of DMF]–0.122 mM Nitro Blue Tetrazolium).

Quantitative immunoscreens were done by growing the cells to mid-log phase in CT, resuspending the cells to 4×10^8, 8×10^7, 1.6×10^7, and 3.2×10^6 cells per ml, spotting 25-μl samples onto nitrocellulose on the suction apparatus, and then screening by the aforementioned procedure.

All of the mutants used in this study were streaked three times for isolated colonies and were rescreened at each step for lack of reactivity with the corresponding MAb.

Standard enzyme-linked immunosorbent assay (ELISA). Standard ELISAs were performed by the procedure of Jemmerson (11), with the following modifications: horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG), affinity purified (no. A-0412, Sigma Chemical Co.), was used as the detecting agent, and purified MAbs were quantified by A_{280} and were diluted with phosphate-buffered saline containing 5% horse serum. A 100-μl sample of diluted antibody per well was used. For the assay, antibody dilutions were incubated overnight with the cells in the microdilution wells.

Cells were prepared for the ELISA as follows. Cultures were grown in liquid CT as described above; cells were deposited on the bottoms of the wells of microdilution plates (Corning) as described previously for submerged fruiting (9). After 24 h of growth, the cell supernatant was removed and the wells were washed once in distilled H_2O for 5 min, washed twice briefly with 0.1% Triton X-100 in phosphate-buffered saline and incubated for 30 min with 200 μl of phosphate-buffered saline containing 5% horse serum. After this treatment, the appropriate antibodies were added to the wells.

The following MAbs were used in the ELISA. MAb 1604 (IgG2a isotype), affinity purified on protein A columns, was provided by Martin Dworkin; MAb 2E5G9 (IgG1 isotype), directed against horse cytochrome *c* and affinity purified on horse cytochrome *c* columns, was provided by Ron Jemmerson; MAb 159-18 (IgG2a isotype), directed against the VP3 envelope glycoprotein of lactate dehydrogenase-elevating virus and affinity purified on protein A columns, was provided by Peter Plagemann; MAbs A9.C6 (IgG2a) and C10.C10 (IgG2b), directed against different epitopes on bovine retinal S protein and affinity purified on Sepharose columns, were provided by Dale Gregerson.

EMS mutagenesis. JZ007 was grown to 2×10^8 cells per ml, and 1 ml of cells was transferred to a 15-ml corex tube. Fifteen microliters of ethyl methanesulfonate (EMS; Sigma) was added to the cells, and the tube was incubated for 2 h at 32°C with frequent intermittent shaking. Four milliliters of CT was added to the tube, and the cells were pelleted at 4,340 × *g* for 10 min. The cells were resuspended in 5 ml of CT, and 0.2-ml samples were transferred to small glass tubes. The tubes were slowly shaken overnight in a moist chamber at 32°C.

Bacteriophage selection and immunoscreening of EMS-induced and spontaneous mutants. Bacteriophage (0.1 ml, 5×10^9 to 5×10^{10} PFU/ml) was added to each tube of EMS-induced mutants and was allowed to adsorb for 30 min at 32°C. Each tube was plated out in 2.5 ml of CTT top agar on a CTT plate and incubated for 5 days at 32°C. This resulted in approximately 100 colonies per plate. Individual colonies were immunoscreened as previously described; antigen-minus colonies were streaked three times for isolated colonies, were rescreened, and then were frozen in 20% glycerol at −70°C. Only 1 mutant per plate was saved to ensure independency.

For spontaneous mutants, JZ007 was grown to approximately 2×10^7 cells per ml. Samples (0.2 ml) of cells were incubated with 0.1 ml of bacteriophage (5×10^9 to 5×10^{10} PFU/ml) for 30 min at 32°C. The cells were plated, screened, and stored as described above.

Polyacrylamide gel electrophoresis, Western blot analysis, and India ink staining. Electrophoresis, Western blot analysis, and India ink staining were carried out as described elsewhere (6).

DNA isolation and restriction, electrophoresis, and Southern blot analysis. *M. xanthus* DNA was isolated, restricted,

and electrophoresed as previously described (15). Nick translation of pBR322::Tn*5* DNA was carried out as described elsewhere (17). Southern blot analysis was done as previously described (22).

RESULTS

Isolation of transposon Tn*5* mutants defective in MAb binding. We created a large collection of independent mutants of *M. xanthus* JZ007 by using transposon mutagenesis. The transposon was delivered into the *M. xanthus* cells by transduction with the suicide phage vector P1::Tn*5* (12). Although this *E. coli* phage adsorbs to *M. xanthus* and injects its DNA, it fails to replicate its DNA in this foreign host or to persist in the latent state as a DNA plasmid (12). However, the transposon Tn*5* can transpose from the injected P1::Tn*5* DNA and insert into the *M. xanthus* genome, causing random mutations (12). We isolated a total of 7,488 independent mutants, using kanamycin resistance encoded by the transposon as a selective genetic marker.

To screen these Tn*5* mutants for their inability to bind MAbs, we first grew each mutant in liquid and spotted samples of cells, in replicate, onto sheets of nitrocellulose. We then screened the replicate nitrocellulose spot blots with different MAbs, using an ELISA. We screened all 7,488 Tn*5* mutants and obtained 6 independent mutants, each lacking a specific epitope recognized by a MAb. Five of the mutants (JZ1612, JZ2482, JZ2712, JZ5630, and JZ7503) lacked reactivity with MAb 1514, a MAb that had been shown previously to recognize a carbohydrate epitope on the O antigen of LPS (6). The other mutant (JZ5966) lacked immunoreactivity with MAb 1604, a MAb which had been shown previously to recognize a protein molecule (9, 10).

We used genetic transduction to prove that the Tn*5* insertions within the mutant strains were the cause of the defect in affinity for the MAb. Generalized transducing lysates made on each of the mutant strains were used to transduce an immunoreactive reference recipient strain. Kanamycin-resistant transductants were selected and then assayed for the capacity to bind MAbs. The majority of the transductants (48 of 48 for JZ1612, 47 of 48 for JZ2482, 48 of 48 for JZ2712, 31 of 36 for JZ5630, 173 of 173 for JZ5966, and 42 of 42 for JZ7503) were nonimmunoreactive with the appropriate MAb. This suggested that the transposons were responsible for the defective phenotype and that each acted as a single mutation in affecting the epitope for antibody binding.

M. xanthus strains may vary in a property which affects the cohesiveness of the cells (21). Three of the original mutants (JZ2712, JZ5630, and JZ5966) were noncohesive in phenotype. The transposons could be transduced from the noncohesive strains JZ5630 and JZ5966 into the reference recipient strain JZ007, producing cohesion-plus transductants. One transductant of each Tn*5* mutant (except JZ2712) was verified to be wild type for the cohesion phenotype, and these strains (JZ7989, JZ8067, JZ8134, JZ8139, and JZ8900) were used for all further studies. This helped to ensure that the transposon mutations were studied in the reference genetic background of JZ007 (Table 1). However, the transposon from the noncohesive strain JZ2712 produced transductants which were noncohesive. This suggested that the transposon caused the defect in cohesion or that it was closely linked to a second mutation causing the defect. One transductant derived from JZ2712 (JZ8065) was used for further studies (Table 1).

We performed Southern analysis on chromosomal DNA from each of the mutant strains to directly confirm, by hybridization, the presence of the transposon Tn*5* DNA. Chromosomal DNA from the wild-type strain and each of the mutant strains was restricted with endonuclease *Eco*RI, which does not cut transposon Tn*5*. The autoradiogram (Fig. 1) showed the presence of a single hybridizable band for each of the mutants. This indicated that each mutant contained only one Tn*5* insertion. All of the hybridizable DNA bands had molecular sizes of between 24 and 45 kilobases. At least four of the bands for the LPS mutants were at unique molecular weights, indicating that at least four of the transposons had inserted in different sites.

Quantitation of the defect in MAb binding. We performed quantitative immunoblot assays to determine the degree of the defect in each of the mutants. Quantitative immunoblots were performed by spotting cells at different dilutions onto the nitrocellulose and then screening the spots with the appropriate MAb. The quantitative spot immunoblots using MAb 1514 showed that the five 1514 mutant strains were at least 100-fold lower in immunoreactivity than the reference strain (Fig. 2). We had shown previously that MAbs 2600, 1733, 1514, 1412, and 783 bound to the O antigen of LPS (6). Three of these MAbs (2600, 1733, and 1412) resembled MAb 1514 in their reactivities on the quantitative spot immunoblots (Fig. 2). MAb 783 bound to two of the mutants, JZ8065 and JZ8067, but not to the other three mutants (Fig. 2). The amount of immunoreactivity of JZ8065 and JZ8067 with MAb 783 varied from experiment to experiment, with the levels fluctuating from that of the wild type to approximately 5-fold less.

We characterized the amount of reactivity with MAb 1514 more precisely by using twofold dilutions of cells in the spot immunoblot assay (data not shown). This indicated that JZ7989, JZ8134, and JZ8139 contained less than 0.2% of the immunoreactivity of the reference strain, JZ8067 had approximately 0.4% of the immunoreactivity of the reference strain, and JZ8065 had approximately 0.8% of the immunoreactivity of the reference strain.

The nonimmunoreactivity of the cell surface antigen (csa) 1604 mutant (JZ8900) also was determined quantitatively (Fig. 3 and 4). By spot blot analysis (Fig. 3), JZ8900 had less than 4% of the immunoreactivity of the reference strain (JZ007) when probed with MAb 1604. Standard ELISA analysis of JZ007 and JZ8900 (Fig. 4) was performed under conditions identical to those found at the initiation of submerged fruiting (9, 10). We observed that purified MAb 1604 bound to reference cells, and binding increased with linear kinetics for antibody concentrations up to approximately 2 ng of MAb 1604 antibody per well. The kinetics of antibody binding suggested that saturation of the antibody-binding sites occurred at antibody concentrations above approximately 10 ng per well (Fig. 4A, inset).

If the average IgG antibody is 150 kilodaltons, then 10 ng of IgG antibody is equivalent to 4×10^{10} antibody molecules. This amount of antibody in a well equals approximately 2,000 antibody molecules per cell (4×10^{10} antibody molecules per 2×10^7 cells) and equals approximately the amount of antibody required to give saturation of binding in this ELISA of MAb 1604 with reference cells.

Figure 4 shows that the csa 1604 mutant (JZ8900) was nonimmunoreactive with MAb 1604 in the ELISA, with no detectable binding of antibody in that range of antibody concentrations (approximately 1 to 10 ng of MAb per well) which gave linear kinetics of binding to the reference cells. At much higher concentrations of antibody we did observe detectable binding of MAb 1604 to the mutant. However, in those concentration ranges of antibody, IgG antibodies

TABLE 1. Bacterial strains[a]

Strain	Genotype	Phenotype								Origin
		Csa 2600	Csa 1733	Csa 1514	Csa 1412	Csa 783	Csa 2254	Csa 1604	ϕ	
ys	Reference	+	+	+	+	+	+	+	s	FB variant
JZ007	Reference	+	+	+	+	+	+	+	s	DK1622
JZ501-JZ7988	JZ007::Tn*5*									P1::Tn*5* × JZ007
JZ1612	JZ007Ω1612 (*lps-1*::Tn*5*)	−	−	−	−	−	+	+	r	P1::Tn*5* × JZ007
JZ2482	JZ007Ω2482 (*lps-2*::Tn*5*)	−	−	−	−	−	+	+	r	P1::Tn*5* × JZ007
JZ2712	JZ007Ω2712 (*lps-3*::Tn*5*) *coh*	−	−	−	−	+	+	+	r	P1::Tn*5* × JZ007
JZ5630	JZ007Ω5630 (*lps-4*::Tn*5*) *coh*	−	−	−	−	+	+	+	r	P1::Tn*5* × JZ007
JZ5966	JZ007Ω5966 (*csa1604*::Tn*5*) *coh*	+	+	+	+	+	+	−		P1::Tn*5* × JZ007
JZ7503	JZ007Ω7503 (*lps-5*::Tn*5*)	−	−	−	−	−	+	+	r	P1::Tn*5* × JZ007
JZ7989	JZ007Ω2482 (*lps-2*::Tn*5*)	−	−	−	−	−	+	+	r	JZ2482 × JZ007
JZ8065	JZ007Ω2712 (*lps-3*::Tn*5*) *coh*	−	−	−	−	+	+	+	r	JZ2712 × JZ007
JZ8067	JZ007Ω5630 (*lps-4*::Tn*5*)	−	−	−	−	+	+	+	r	JZ5630 × JZ007
JZ8134	JZ007Ω1612 (*lps-1*::Tn*5*)	−	−	−	−	−	+	+	r	JZ1612 × JZ007
JZ8139	JZ007Ω7503 (*lps-5*::Tn*5*)	−	−	−	−	−	+	+	r	JZ7503 × JZ007
JZ8441	*lps-6*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8442	*lps-7*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8446	*lps-8*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8447	*lps-9*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8448	*lps-10*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8449	*lps-11*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8450	*lps-12*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8451	*lps-13*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8452	*lps-14*			−		+/−	+		r	JZ007 EMS, Mx8^r
JZ8453	*lps-15*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8454	*lps-16*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8455	*lps-17*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8456	*lps-18*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8457	*lps-19*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8458	*lps-20*			+/−		−	+		r	JZ007 EMS, Mx8^r
JZ8459	*lps-21*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8460	*lps-22*			+/−		+/−	+		r	JZ007 EMS, Mx8^r
JZ8461	*lps-23*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8462	*lps-24*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8463	*lps-25*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8464	*lps-26*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8465	*lps-27*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8466	*lps-28*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8467	*lps-29*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8468	*lps-30*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8469	*lps-31*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8470	*lps-32*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8471	*lps-33*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8472	*lps-34*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8473	*lps-35*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8474	*lps-36*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8475	*lps-37*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8476	*lps-38*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8477	*lps-39*			−		+/−	−		r	JZ007 EMS, Mx8^r
JZ8478	*lps-40*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8479	*lps-41*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8480	*lps-42*			+/−		+/−	+		r	JZ007 EMS, Mx8^r
JZ8481	*lps-43*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8482	*lps-44*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8483	*lps-45*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8484	*lps-46*			−		−	+		r	JZ007 EMS, Mx8^r
JZ8485	*lps-47*			−		−	+		r	JZ007 spont., Mx8^r
JZ8486	*lps-48*			−		−	+		r	JZ007 spont., Mx8^r
JZ8487	*lps-49*			−		+/−	+		r	JZ007 spont., Mx8^r
JZ8488	*lps-50*			−		−	+		r	JZ007 spont., Mx8^r
JZ8489	*lps-51*			−		−	+		r	JZ007 spont., Mx8^r
JZ8490	*lps-52*			−		−	+		r	JZ007 spont., Mx8^r
JZ8491	*lps-53*			−		−	+		r	JZ007 spont., Mx8^r
JZ8492	*lps-54*			−		−	+		r	JZ007 spont., Mx8^r
JZ8493	*lps-55*			+		+	−		r	JZ007 EMS, Mx1^r

Continued on following page

TABLE 1—*Continued*

Strain	Genotype	Phenotype								Origin
		Csa 2600	Csa 1733	Csa 1514	Csa 1412	Csa 783	Csa 2254	Csa 1604	Φ	
JZ8494	*lps-56*			+		+	−		r	JZ007 EMS, Mx1[r]
JZ8495	*lps-57*			−		−	+		r	JZ007 EMS, Mx1[r]
JZ8496	*lps-58*			−		−	+		r	JZ007 EMS, Mx1[r]
JZ8497	*lps-59*			+		+	−		r	JZ007 EMS, Mx1[r]
JZ8498	*lps-60*			−		−	+		r	JZ007 spont., Mx1[r]
JZ8499	*lps-61*			−		−	+		r	JZ007 spont., Mx1[r]
JZ8790	JZ007Ω5630 (*lps-4*::Tn*5*) *coh*	−	−	−	−	+	+		r	JZ5630 × JZ007
JZ8900	JZ007Ω5966 (*csa1604*::Tn*5*)	+	+	+	+	+	+	−		P1::Tn5 × JZ007

[a] Ω, Site of Tn*5* insertion; ::Tn*5*, a Tn*5* insertion; Tn*5*, transposon Tn*5*; *lps*, lipopolysaccharide; *coh*, cohesion; *dsp*, dispersive; Csa, cell surface antigen; Mx1, bacteriophage Mx1; Mx8, bacteriophage Mx8; φ, phage; r and s, resistant or sensitive, respectively, to the phage listed in the origin column; +, presence; −, absence; +/−, reduced level. Blank spaces in the phenotype column represent untested phenotypes. EMS, Ethyl methanesulfonate-induced mutation; spont., spontaneous mutation.

directed against horse cytochrome *c*, bovine retinal S protein, and the lactate dehydrogenase-elevating virus envelope glycoprotein VP-3 all showed binding to the reference strain JZ007 (Fig. 4B) and to the csa 1604 mutant (JZ8900) (data not shown). These experiments with heterologous antibodies showed that antibody binding at very high concentrations of antibody can be nonimmunospecific. We therefore conclude that the low level of binding of MAb 1604 to the csa 1604 mutant JZ8900 is nonimmunospecific when MAb 1604 is present at a high concentration.

Western blot analysis of the transposon Tn*5* mutants defective in MAb binding. The loss of immunoreactivity on a spot blot could be due to either the loss of the immunoreactive epitope or to a masking of that epitope. These two possibilities could be distinguished by Western blot analysis of whole-cell lysates, since electrophoresis may unmask epitopes by separating non-covalently linked molecules. Western blot analysis of whole-cell lysates (Fig. 5) showed that each of the LPS Tn*5* mutants was still nonreactive with MAb 1514. MAb 783 bound to LPS from only the two mutants which possessed reactivity in the spot immunoblots. We conclude that masking was not responsible for the original defect in MAb affinity in these mutants.

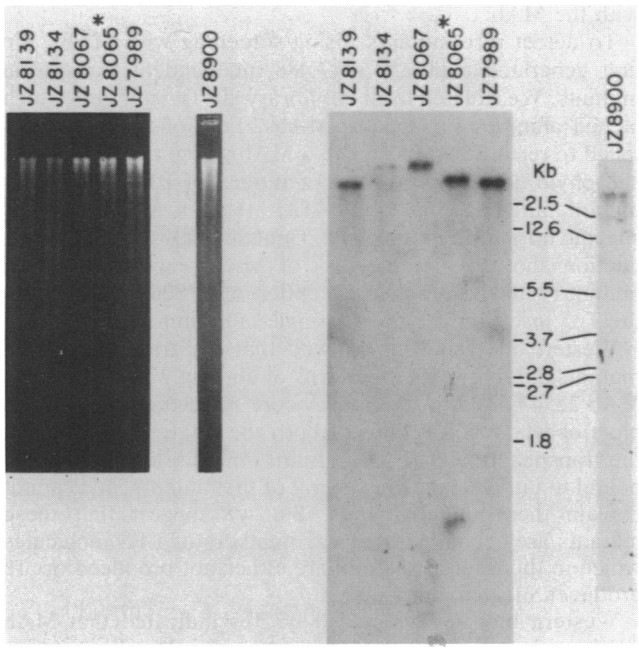

FIG. 1. A 0.5% agarose gel and a 0.7% agarose gel (left) of *Eco*RI-restricted DNA from the transposon Tn*5* mutant strains. Corresponding autoradiographs (right) of Southern blots of the gels probed with radiolabeled pBR322::Tn*5* DNA. The * indicates that JZ8065* may contain a second mutation which affects the cohesive properties of the cell. Molecular sizes (in kilobases) are indicated.

MAb 2254, which reacts with the core of LPS (6), recognized all of the LPS mutants on a spot immunoblot (data not shown). A Western blot (Fig. 5) showed that MAb 2254 bound to the low-molecular-weight lipid A core band of the wild type and each of the five LPS Tn*5* mutants; it also bound to several unique bands in some of the mutants. MAb 2254 also bound to a low-molecular-weight singlet band in JZ8134, a low-molecular-weight doublet in JZ8139 (containing the singlet band of JZ8134), and a low-molecular-weight doublet (the same as JZ8139) and a slightly higher-molecular-weight singlet in JZ7989.

Western blot analysis of the csa 1604 mutant strain (Fig. 6) showed that the mutant was missing the multiple high-molecular-weight bands of immunoreactivity that were present in the reference strain. We conclude that masking was not responsible for the original defect in binding MAb 1604 in this mutant.

Isolation of bacteriophage-resistant mutants defective in MAb binding. Since LPS acts as a bacteriophage receptor in many species of bacteria, LPS mutants of *M. xanthus* could be resistant to certain myxophages. We discovered that the LPS Tn*5* mutants were altered in their sensitivities to several of the known myxophages. None of these Tn*5* mutants adsorbed phages Mx8 (14), while the reference strain adsorbed more than 95% of this bacteriophage under identical conditions. This suggests that LPS could be the receptor for bacteriophage Mx8 in *M. xanthus* and that phage resistance could be used as a selective technique for isolating LPS mutants.

We used Mx1 (3), a virulent myxophage, and Mx8riv1, a clear-plaque derivative of Mx8a (15), to select for phage-resistant colonies of untreated and EMS-mutagenized JZ007. A large number of phage-resistant strains were isolated. These strains were subsequently screened for the loss of reactivity with either MAb 1514 or 2254. Approximately 5% of the Mx1-resistant colonies and 25% of the Mx8-resistant colonies were nonreactive with one or the other of the MAbs. Fifty-six independent phage-resistant mutants were isolated and characterized (Table 1). From this set, all of the

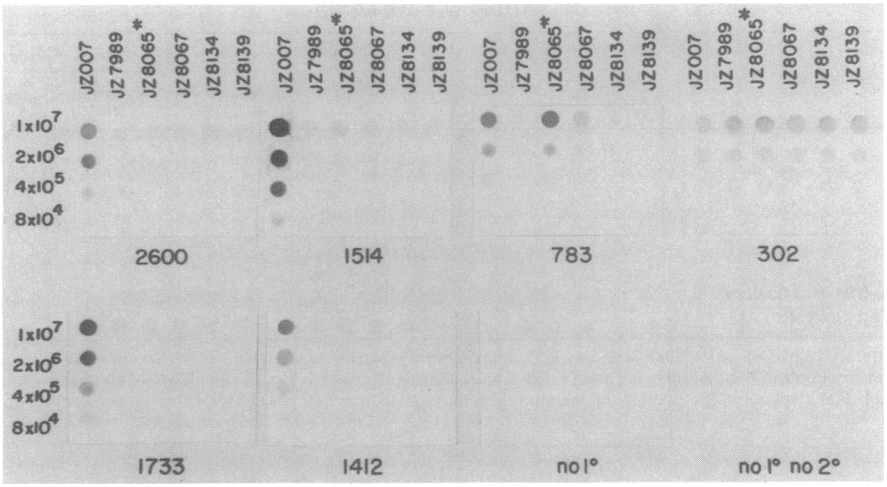

FIG. 2. Quantitative spot immunoblots of eight replicate nitrocellulose filters containing the reference strain (JZ007) and the transposon Tn*5* mutant strains (JZ7989, JZ8065*, JZ8067, JZ8134, and JZ8139). The numbers of bacteria per spot are indicated on the left. The number beneath each replicate immunoblot indicates the MAb that was used as a probe in that immunoblot. no 1°, No anti-myxobacterial MAb used in the ELISA; no 1° no 2°, neither the anti-myxobacterial MAb nor the enzyme-conjugated antibody used in the ELISA. The * indicates that JZ8065* may contain a second mutation which affects the cohesive properties of the cell.

Mx8-resistant colonies (JZ8441, JZ8442, and JZ8446 to JZ8492) were reactive with MAb 2254 and nonreactive with MAb 1514. This indicated that these mutants had altered or missing O antigens. Both of the spontaneous Mx1-resistant mutants (JZ8498 and JZ8499) and two of the EMS-mutagenized Mx1-resistant mutants (JZ8495 and JZ8496) were reactive with MAb 2254 and nonreactive with MAb 1514, indicating that these mutants had altered or missing O antigens. Three of the EMS-mutagenized Mx1-resistant mutants (JZ8493, JZ8494, and JZ8497) were reactive with MAb 1514 and nonimmunoreactive with MAb 2254, indicating that these mutants retained the O antigen but were missing an epitope on the LPS core.

DISCUSSION

MAbs have been isolated which recognize specific molecules on the cell surface of *M. xanthus* (8, 9). These MAbs have been used as probes to identify cell surface molecules which may be critical for cell interactions or cell signaling during development (8–10). By virtue of their binding to specific epitopes on cell surface molecules and blocking cell contacts or cell signaling, these MAbs could be used as specific inhibitors in biological assays for cell behavior (10).

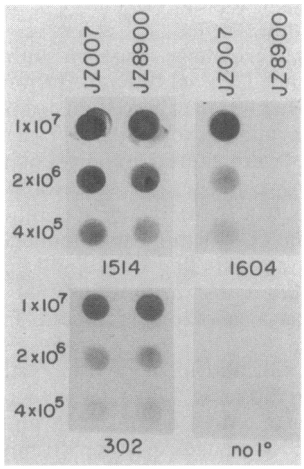

FIG. 3. Quantitative spot immunoblots of four replicate nitrocellulose filters containing the reference strain (JZ007) and the transposon Tn*5* mutant strain JZ8900. The numbers of bacteria per spot are indicated on the left. The number beneath each replicate immunoblot indicates the MAb that was used as a probe in that immunoblot.

We have used these MAbs in genetic strategies to isolate *M. xanthus* mutants which lack particular cell surface antigens. This genetic approach should be complementary to the immunological approach because a mutant missing a particular antigen should behave like the wild-type strain inhibited with the MAb.

To detect rare mutants, using screening with MAbs, we first generated a library of 7,488 independent transposon mutants. We isolated from our library six Tn*5* mutants which lacked affinities for specific MAbs. Five of these mutants failed to react with MAb 1514, a MAb which is specific for a carbohydrate epitope on the O antigen of LPS. The other mutant failed to react with MAb 1604, a MAb which is specific for a protein molecule. Genetic analysis using transduction showed that the loss of MAb reactivity in each mutant (with the possible exception of JZ8065) was due to the Tn*5* insertion acting as a single mutation.

Western blot analysis showed that the transposon LPS mutants differed in their patterns of binding with MAb 2254. MAb 2254 bound to the lipid A core molecules on Western blots of whole-cell lysates of both the reference strain and the transposon LPS mutant strains. In addition, MAb 2254 bound to unique bands in several of the mutants. We cannot explain these unique bands, but we suggest that these mutants are accumulating large numbers of LPS molecules which in the reference strain are either not produced or are produced in small amounts.

Western blots of whole-cell lysates indicated that MAb 783 bound to the LPS stepladder of molecules observed for the Tn*5* mutants JZ8065 and JZ8067. Since these stepladder molecules retained immunoreactivity with MAb 783 but not with MAb 1514, we reason that the epitope for MAb 1514 could be a side-chain carbohydrate on the O antigen of the wild-type LPS molecule. The MAb 783-binding site could be

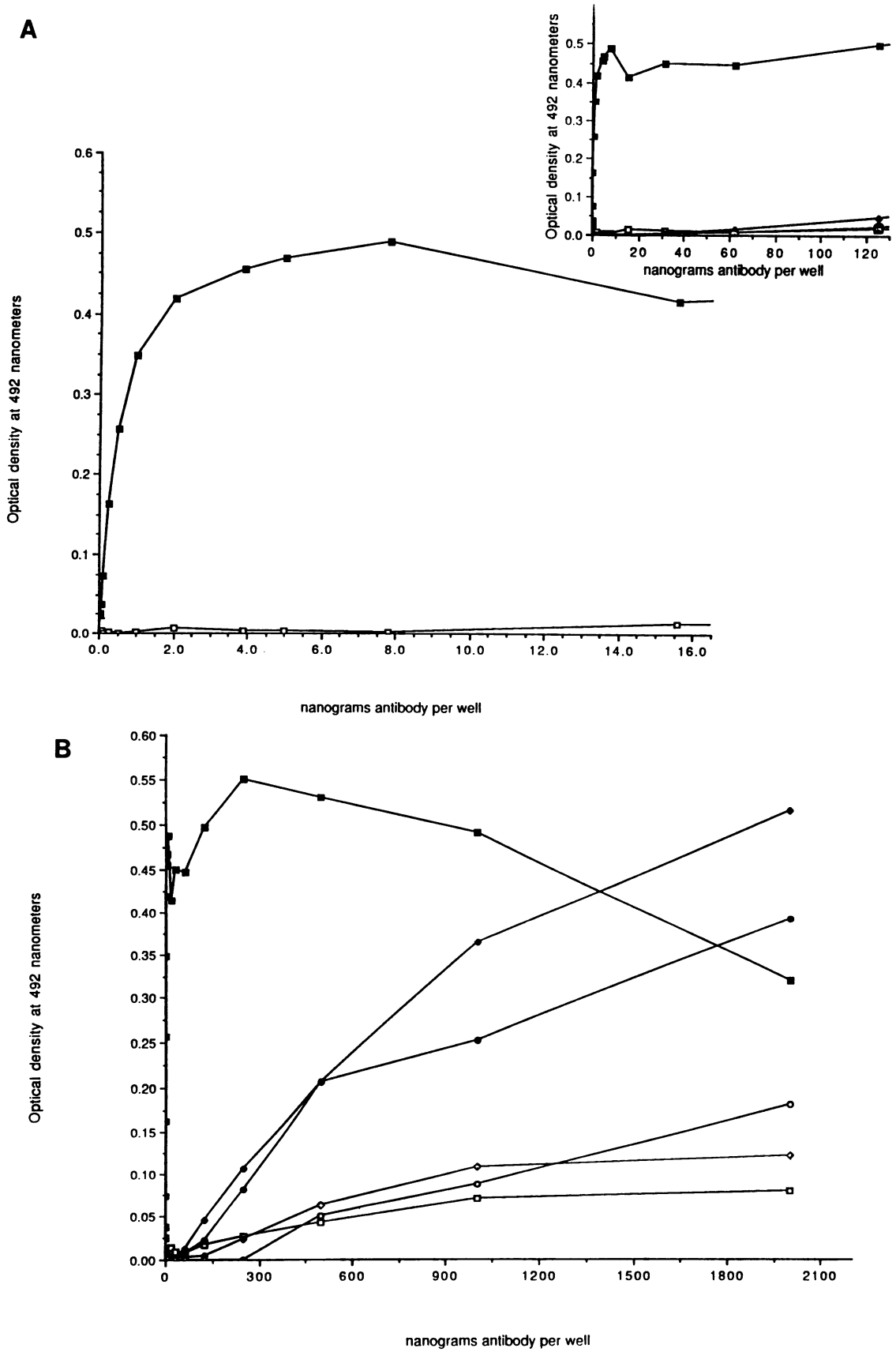

FIG. 4. Standard curves comparing the optical density readings in ELISAs of strains JZ007 and JZ8900 with increasing amounts of antibody added per well. (A) Optical density reading in ELISAs of JZ007 and JZ8900 reacted with MAb 1604 in the range of 0 to 16 ng of antibody per well. Inset: identical to panel A, except data are shown over a greater range of antibody added per well. (B) Reactivities of various control MAbs in ELISAs with JZ007 and reactivity of MAb 1604 with JZ007 and JZ8900, in the range of 0 to 2100 ng of antibody per well. Symbols: ■, JZ007 versus MAb 1604; □, JZ8900 versus MAb 1604; ●, JZ007 versus MAb 159-18; ◆, JZ007 versus MAb A9.6C; ◇, JZ007 versus MAb C10.C10; ○, JZ007 versus MAb 2E5G9.

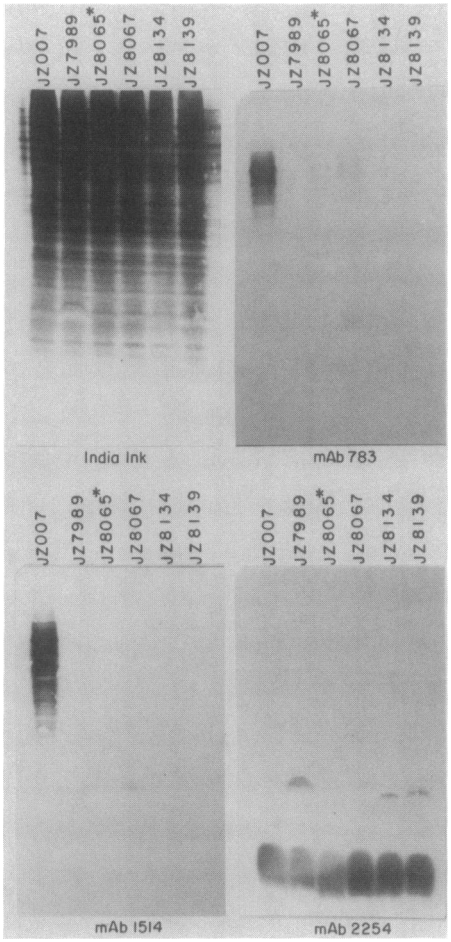

FIG. 5. Four identical Western blots of whole-cell lysates (8.75 × 10^7 cells per lane) of the reference strain (JZ007) and the transposon Tn*5* mutant strains (JZ7989, JZ8065*, JZ8067, JZ8134, and JZ8139) electrophoresed on a 15 to 20% linear polyacrylamide gradient gel at 20 mA for 16 h. The upper left-hand panel represents an India ink stain while the other three panels represent Western blots using MAbs (783, 1514, and 2254) specific for LPS as probes. The * indicates that JZ8065* may contain a second mutation which affects the cohesive properties of the cell.

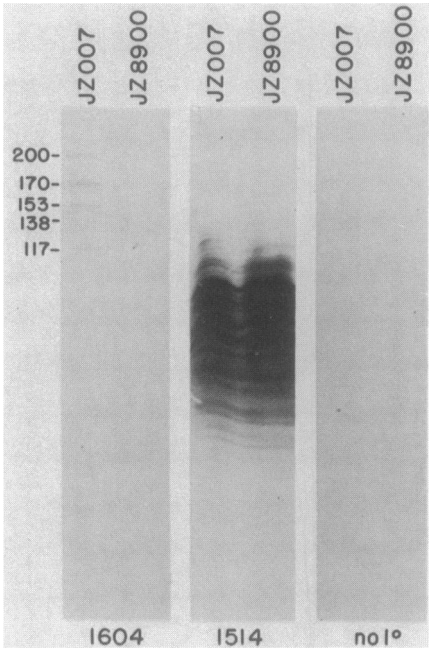

FIG. 6. Three identical Western blots of whole-cell lysates (8.75 × 10^7 cells per lane) of the reference strain (JZ007) and the transposon Tn*5* mutant strain JZ8900 electrophoresed on a 7.0% polyacrylamide gel at 40 mA overnight. Cells were grown for 24 h in CTT medium on tissue culture flask bottoms (Costar) as described for submerged fruiting (9) prior to harvesting and preparation for polyacrylamide gel electrophoresis. The number beneath each replicate Western blot indicates the MAb that was used as a probe in that blot.

either a side-chain carbohydrate of the O antigen or a backbone carbohydrate of the O antigen.

The altered sensitivity of the Tn*5* LPS mutants to myxophage Mx8 indicated that LPS may be the Mx8 receptor. Phage adsorption experiments confirmed that these mutants were resistant to Mx8 at the level of adsorption. We conclude that LPS is probably the receptor for bacteriophage Mx8. Alternatively, a different molecule may be the bacteriophage receptor if each of the five independent Tn*5* mutations causes a secondary effect inhibiting phage adsorption. Mutations in LPS biosynthesis could have secondary effects on membrane stability or particular membrane proteins. Mutations in LPS biosynthesis also could have secondary effects on the biosynthesis of exopolysaccharides. Adsorption experiments have not been performed for bacteriophage Mx1; whether myxophage Mx1 utilizes LPS as a receptor is unknown.

The discovery of phage resistance in the original LPS mutants led to the prediction that selection of phage-resistant strains could be a strategy for isolating new LPS mutants. This proved to be correct, and we were able to isolate a large number of new mutants altered in their affinities for the LPS-specific MAbs. These mutants had properties different from those previously reported for Mx8*cp*2 lysogens (19). Western analysis of the 56 phage-resistant mutants, using the six MAbs which bind to LPS epitopes, could provide further insight into the structure of LPS from *M. xanthus*.

Several of the Mx1-resistant LPS mutants bound MAb 1514 but not MAb 2254. Since these mutants retained reactivity with the MAb specific for O antigen, they must contain the backbone core carbohydrates. This indicates that MAb 2254 binds to a side-chain carbohydrate epitope of the core.

To determine whether a particular Tn*5* mutant was completely devoid of affinity for its respective MAb, we tested quantitative dilutions of that mutant in a spot immunoblot assay. We could detect binding of MAb 1514 to a spot of reference cells, even if these were diluted by 500-fold. This suggested that a mutant with 500-fold less reactivity than the reference cells still could be detected as positive by this assay. Therefore, we define 0.2% (1/500) of the original activity to be the limit of resolution of the assay. If we assume that each cell contains the same number of MAb-binding sites, then the minimum number of sites which could be detected is 0.2% of the original number of sites. Scatchard analysis previously had shown that there were 54,000 MAb 1514-binding sites per reference bacterium (B. Jarvis, Ph.D. thesis, University of Minnesota, Minneapolis, 1988). Therefore, the limit of resolution of our assay for MAb 1514-binding sites is 0.2% of 54,000, or approximately 100 sites

per cell. This means that we could not assess quantitatively the extremely low immunoreactivities of mutants containing either fewer than 0.2% (100) of the MAb 1514-binding sites or MAb 1514-binding sites containing less than 0.2% of the normal affinity.

We could detect binding of MAb 1604 to a spot of reference cells, even if the cells were diluted 25-fold. By identical reasoning, this indicated that the minimum number of sites per cell which we could detect at the limit of resolution of this assay was 4% (1/25) of the original number. Again, we assume that all cells are equal in MAb 1604 binding. Scatchard analysis previously had shown that there were 2,400 MAb 1604-binding sites per reference cell (Jarvis, Ph.D. thesis). Therefore, the limit of resolution of our assay for MAb 1604-binding sites is 4% of 2,400, or approximately 100 sites per cell. This is consistent with the previously detected limit of resolution for MAb 1514. This means that we could not assess the degree of immunoreactivity of a mutant containing either very low levels of MAb 1604-binding sites (fewer than 100 sites per cell) or less than 4% of the normal affinity for MAb 1604.

Three of the LPS transposon mutants and the 1604 transposon mutant had no specific MAb-binding activity (1514 or 1604, respectively) which could be detected by our quantitative assay. This means that these mutants contain either fewer than 100 MAb-binding sites (1514 or 1604, respectively) per cell or less than 0.2 or 4.0% of the affinity for the MAb (1514 or 1604, respectively).

The other two LPS transposon mutants had approximately 0.4 and 0.8% of their MAb 1514-binding sites. There are two possible explanations for this low but detectable level of reactivity. First, the Tn*5* insertion may reside in the distal end of a gene, allowing synthesis of an altered gene product with decreased activity. Second, the Tn*5* insertion may reside outside the gene but cause decreased gene expression.

The genetic approach using MAbs to screen for nonimmunoreactive mutants has enabled us to isolate mutants lacking specific cell surface molecules without having any prior knowledge about the function(s) of these molecules in biological behavior. We observed that the transposon mutant defective in the MAb 1604 epitope was normal in biological assays for developmental behavior (formation of fruiting bodies on clone fruiting plates [18] or in submerged-fruiting assays). A mutant with less than 4% of a normal epitope might be defective in phenotype, if that epitope is critical for developmental behavior in these assays. We therefore conclude that the cell surface antigen defined by the MAb 1604 epitope probably is not critical for development under these experimental conditions. In another study (7), we report on the biological behavior of the Tn*5* LPS mutants and show that the O-antigen mutants are defective in motility but not dramatically altered in development. We also show that the LPS core mutants are developmentally aberrant, failing to form multicellular fruiting bodies.

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LITERATURE CITED

1. **Blake, M. S., K. H. Johnson, G. J. Russel-Jones, and E. C. Gotschlich.** 1984. A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. Anal. Biochem. **136**:175–179.
2. **Bretscher, A. P., and D. Kaiser.** 1978. Nutrition of *Myxococcus xanthus*, a fruiting myxobacterium. J. Bacteriol. **133**:763–768.
3. **Burchard, R. P., and M. Dworkin.** 1966. A bacteriophage for *Myxococcus xanthus*: isolation, characterization, and relation of infectivity to host morphogenesis. J. Bacteriol. **91**:1305–1313.
4. **Campos, J. M., J. Geisselsoder, and D. R. Zusman.** 1978. Isolation of bacteriophage Mx4, a generalized transducing phage for *Myxococcus xanthus*. J. Mol. Biol. **119**:167–178.
5. **Dworkin, M.** 1962. Nutritional requirements for vegetative growth of *Myxococcus xanthus*. J. Bacteriol. **84**:250–257.
6. **Fink, J. M., and J. F. Zissler.** 1989. Characterization of lipopolysaccharide from *Myxococcus xanthus* by use of monoclonal antibodies. J. Bacteriol. **171**:2028–2032.
7. **Fink, J. M., and J. F. Zissler.** 1989. Defects in motility and development of *Myxococcus xanthus* lipopolysaccharide mutants. J. Bacteriol. **171**:2042–2048.
8. **Gill, J., E. Stellwag, and M. Dworkin.** 1985. Monoclonal antibodies against cell-surface antigens of developing cells of *Myxococcus xanthus*. Ann. Microbiol. (Paris) **136A**:11–18.
9. **Gill, J. S., and M. Dworkin.** 1986. Cell surface antigens during submerged development of *Myxococcus xanthus* examined with monoclonal antibodies. J. Bacteriol. **168**:505–511.
10. **Gill, J. S., B. W. Jarvis, and M. Dworkin.** 1987. Inhibition of development in *Myxococcus xanthus* by monoclonal antibody 1604. Proc. Natl. Acad. Sci. USA **84**:4505–4508.
11. **Jemmerson, R.** 1987. Multiple overlapping epitopes in the three antigenic regions of horse cytochrome c^1. J. Immunol. **138**: 213–219.
12. **Kuner, J. M., and D. Kaiser.** 1981. Introduction of transposon Tn*5* into *Myxococcus* for analysis of developmental and other nonselectable mutants. Proc. Natl. Acad. Sci. USA **78**:425–429.
13. **Luria, S., and M. Delbruck.** 1942. Interference between bacterial viruses. Arch. Biochem. Biophys. **1**:111–141.
14. **Martin, S., E. Sodergren, T. Masuda, and D. Kaiser.** 1978. Systematic isolation of transducing phages for *Myxococcus xanthus*. Virology **88**:44–53.
15. **Orndorff, P. E., E. Stellwag, T. Starich, M. Dworkin, and J. Zissler.** 1983. Genetic and physical characterization of lysogeny by bacteriophage MX8 in *Myxococcus xanthus*. J. Bacteriol. **154**:772–779.
16. **Panasenko, S. M.** 1985. Methylation of macromolecules during development in *Myxococcus xanthus*. J. Bacteriol. **164**:495–500.
17. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
18. **Rosenberg, E. (ed.).** 1984. Myxobacteria: development and cell interactions. Springer-Verlag, New York.
19. **Ruiz-Vázquez, R., and F. J. Murillo.** 1984. Abnormal motility and fruiting behavior of *Myxococcus xanthus* bacteriophage-resistant strains induced by a clear-plaque mutant of bacteriophage Mx8. J. Bacteriol. **160**:818–821.
20. **Schoonejans, E., D. Expert, and A. Toussaint.** 1987. Characterization and virulence properties of *Erwinia chrysanthemi* lipopolysaccharide-defective ϕEC2-resistant mutants. J. Bacteriol. **169**:4011–4017.
21. **Shimkets, L. J.** 1986. Correlation of energy-dependent cell cohesion with social motility in *Myxococcus xanthus*. J. Bacteriol. **166**:837–841.
22. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.