Characterization of Egg Yolk Antibodies for Detection and Quantification of *Selenomonas ruminantium* by Using an Enzyme-Linked Immunosorbent Assay

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The specificity of polyclonal antibodies prepared against strains of *Selenomonas ruminantium*, the effect of assay conditions, and quantification of individual strains in mixed-cell suspensions of selenomonad strains were examined in this study. Whole-cell suspensions were prepared with pure cultures of *S. ruminantium* PC18, HD₄, GA192, and D. Each cell suspension was injected into a Leghorn laying hen, and polyclonal antibodies were harvested from eggs laid in week 3 or 7 following initial immunization. Antibodies made to the *S. ruminantium* strains readily discerned the homologous strain from the heterologous strains. Cross-reactivity among antibodies and the heterologous *S. ruminantium* strains ranged from 5 to 26%. Among non-*S. ruminantium* species, cross-reactivity of *S. ruminantium* antibodies was greatest with *Selenomonas sputigena* (3 to 34%) and *Succinivibrio dextrinosolvens* (0 to 37%). Antibodies made to strains GA192 and D were used to quantify a mixture of the two strains. Both antibodies responded to graded concentrations of the homologous antigen in the biculture mixtures in accord with the change in the direct cell counts for each strain (strain D, $R^2 = 0.92$; strain GA192, $R^2 = 0.90$). This enzyme-linked immunosorbent assay enabled concurrent and accurate quantification of two strains of *S. ruminantium* subsp. *ruminantium* in a mixed-cell suspension with a precision of much less than 1 order of magnitude.

Selenomonas ruminantium is a predominant ruminal bacterial species and is easily recognizable because of its crescent shape, tuft of flagella on the concave side, and tumbling motility. Its distinct morphology has enabled researchers to assess its importance in a variety of diets via direct counts in ruminal fluid (9, 24, 25) or by isolation and presumptive identification (6, 7, 14, 22). Tiwari et al. (23) devised a selective medium utilizing mannitol as the sole carbon and energy source. Culture techniques require 3 to 4 days for colonies to develop, and then further nutritional characterization is needed for taxonomic placement at the subspecies level. None of the above techniques allow for immediate identification or quantification beyond the species level and certainly not of individual strains.

Serological techniques have been used to detect individual strains or species of selenomonads (11, 12). Combining the enzyme-linked immunosorbent assay (ELISA) with species-specific antibodies has made it possible to detect ruminal organisms and potentially quantitate them in mixed culture (1, 10, 15). Ricke et al. (16) found that egg yolk antibodies produced 2 to 7 weeks postimmunization were an expedient source of polyclonal antibodies and used them in an ELISA to detect ruminal bacterial genera and species. Antibodies produced against *S. ruminantium* D were highly species specific, suggesting that strain-specific polyclonal antibodies could potentially be produced from this source. Therefore, we developed antibodies against representative *S. ruminantium* strains that had been separated at the subspecies level based on their ability to ferment lactate (2, 4). In addition, one strain was grown in two media to examine the influence

of the complexity of the antigen growth medium on antibody titer and specificity. Finally, we selected two antigen-antibody combinations to attempt to quantify two strains in a biculture.

MATERIALS AND METHODS

Organisms and culture maintenance. Bovine strains D, GA192, GA31, HD₄, and PC18 of *S. ruminantium* were used as antigens. GA192 and PC18 are the type strains for *S. ruminantium* subsp. *ruminantium* and *lactilytica*, respectively. Ruminal strains for cross-reactivity studies included *Bacteroides ruminicola* subsp. *brevis* B₄, *Butyrivibrio fibrisolvens* D1, *Megasphaera elsdenii* T81, and *Succinivibrio dextrinosolvens* 22B and 24. The above strains were obtained from M. P. Bryant (Department of Animal Science, University of Illinois, Urbana). *Streptococcus bovis* JB1 was obtained from J. B. Russell (Department of Animal Science, Cornell University, Ithaca, N.Y.). *Selenomonas sputigena* ATCC 35185 and *Lactobacillus plantarum* ATCC 14917 were obtained from the American Type Culture Collection (Rockville, Md.). *Salmonella typhimurium* TA831 was obtained from S. Kustu (Department of Microbiology and Immunology, University of California, Berkeley). Culture maintenance and confirmation of culture purity have been described previously (16). The anaerobic technique (3) was used for all culture manipulations. Two culture media were utilized. The rich, ruminal fluid medium (RF medium) contained the following per liter: NH₄Cl, 10 mmol; glucose, 15 mmol; cellobiose, 7.5 mmol; soluble starch, 1 g; Trypticase, 1 g; casein, 1 g; DL-methionine, 3 mmol; Pfennig metals (17), 10 ml; mineral 1 (17), 75 ml; mineral 2 (17), 75 ml; volatile fatty acids (16), 10 ml; B vitamins (17), 100 ml; hemin, 0.15 µmol; clarified ruminal fluid, 300 ml; Na₂CO₃, 4 g; and cysteine hydrochloride · H₂O, 1.4 mmol. The defined medium (DF medium) contained the following ingredients at the

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same concentration per liter as indicated for the RF medium unless otherwise indicated: NH_4Cl ; glucose; Pfennig metals; mineral 3 (17), 50 ml; volatile fatty acids; B vitamins; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 35 μmol ; Na_2CO_3 ; and cysteine hydrochloride $\cdot \text{H}_2\text{O}$.

Growth determination. For evaluation of subspecies, the indicated carbon and energy sources were included in DF medium (containing 0.05% yeast extract) at a concentration of 15 mM hexose equivalent. All cultures were adapted to the carbon and energy sources by at least three transfers in the DF medium containing that source. No growth was detected beyond the first transfer in DF medium without a carbon source. Culture growth was monitored as A_{600} with a light path length of 10 mm.

Protein assays and bacterial adherence to polystyrene wells. Adherence of whole bacterial cells to wells of polystyrene microtiter plates was assessed by assaying the protein which remained bound in wells. One hundred microliters of whole-cell suspensions (containing approximately 5 μg of protein per ml) in carbonate buffer (50 mM, pH 9.6 [16]) was added to duplicate wells of a microtiter plate (Flow Laboratories, Inc., McLean, Va.), as was done routinely for ELISA evaluation of antibodies. Following overnight incubation at 4°C, wells were emptied and rinsed three times with phosphate-buffered saline (pH 7.5). Ten microliters of carbonate buffer was added to each rinsed well. Bovine serum albumin (fraction V bovine albumin; Sigma Chemical Co., St. Louis, Mo.) dissolved in carbonate buffer was used as the protein standard. Aliquots of 10 μl which contained 0 to 1,000 ng of bovine serum albumin were added to duplicate wells. All wells received 200 μl of bicinchoninic acid assay reagent (19), and the reaction was allowed to proceed for 2 h at 22°C. A_{562} was determined with a plate reader (E1 310 Autoreader; Bio-Tek, Burlington, Vt.). All protein determinations were repeated on a second microtiter plate. The average coefficient of variation for all duplicate determinations was 15%.

Immunological analysis. Whole cells utilized for both immunization and ELISAs were prepared from late-log- or early-stationary-phase cultures and were rinsed and centrifuged ($14,500 \times g$) three times with phosphate-buffered saline following fixation with 8% formal-saline (17). Since microbial contaminants grew with prolonged refrigerated storage, cells were stored as a frozen suspension in phosphate-buffered saline containing 0.01% thimerosal (Sigma). Five Leghorn laying hens were immunized as described by Ricke et al. (16) with selenomonad strains GA192, HD₄, PC18, and D. Cell suspensions (0.52 ± 0.07 mg of protein per ml) of these strains grown in DF medium and PC18 also grown in RF medium were injected, with one hen receiving one cell suspension. Every egg laid, including one prior to initial injection (representing preimmunization background), was collected, but only eggs laid in weeks 3 and 7 following initial immunization were utilized. Egg yolk antibodies were harvested, and ELISAs were conducted (16). The ELISA reaction was stopped after 1 h with 4 N H_2SO_4 , and the extent of the reaction was quantified as A_{490} with a plate reader. Heterologous reactivities were calculated as heterologous A_{490} minus day 0 heterologous A_{490} divided by the result of homologous A_{490} minus homologous day 0 A_{490} . The resulting value was multiplied by 100 and given as cross-reactivity percentage.

Quantification of selenomonad strains. Quantification of population sizes for strains D and GA192 in a biculture cell suspension was performed by direct microscopic counts and ELISA. These strains were chosen because they are members of the same subspecies, antibody titers against them

were high with relatively low cross-reactivity, and they are morphologically distinguishable because GA192 is much larger. Strain D was (mean $\pm$ standard error of the mean) 1 ± 0 by 3.1 ± 0.2 μm , and GA192 was 1.3 ± 0.1 by 5.7 ± 0.5 μm . Direct microscopic counts were made with a Petroff-Hausser counting chamber (Arthur H. Thomas Co., Philadelphia, Pa.) as described by Koch (13). Counts prior to admixing the cultures were 8.05×10^7 cells per ml for D and 2.75×10^7 cells per ml for GA192. Eleven mixtures of the two strains were prepared beginning with 100:0 (D:GA192), followed by substitutions of 10 U. Aliquots of 100 μl of each mixture were added to duplicate wells. Antibodies to strain D were obtained from a week 3 egg and diluted 1,600-fold, while GA192 antibodies from a week 7 egg were diluted 100-fold. ELISAs were conducted with these antibodies, and direct counts were determined for each biculture mixture.

Statistical analysis. The total sum of squares for bacterial adherence to polystyrene wells was partitioned among bacterial strain, replicate run, and error. The total sum of squares for cross-reactivity of selenomonad antibodies with heterologous bacterial species was partitioned among antibody, bacterial species, antibody $\times$ species, and error. Before the analysis of variance was conducted, all cross-reactivity percentages were converted to decimals and transformed to the arc sine of the square root of the decimal (20). Due to the presence of 100% values for the homologous reactions, transformation did not reduce the significant skewness of the data ($P < 0.05$) but it did in most cases improve the normality of the plots for the data remaining after the values for homologous reactions were excluded. Therefore, the transformation was retained in all of the cross-reactivity comparisons. If significant ($P < 0.05$) sources of variation were found, multiple comparisons between treatments were made by the least significant difference method (8).

RESULTS

Confirmation of subspecies. Early classifications of *S. ruminantium* were based on nutritional characterizations (2, 4). These nutritional tests were conducted again here to assure that the criteria utilized earlier for strain and subspecies identification were repeatable. The use or nonuse of carbon and energy sources matched previous descriptions for all strains.

Cell adsorption to titer plates. Adsorption of bacterial cells onto the titer plates was examined to determine whether adsorption among strains was quantitatively similar. This was a major consideration when comparing cross-reactivities among heterologous antigen-antibody combinations. Adsorption by the cell suspensions was determined by adding 500 ng of cell suspension protein to microtiter plate wells. Bovine serum albumin added to similar wells served as the protein standard. The standard curves for duplicate plates were consistent ($R^2 = 98.5\%$, 10 points; $R^2 = 97.1\%$, 7 points) over a range of 0 to 1,000 ng of bovine serum albumin per well.

Whole-cell protein adherence to polystyrene wells of microtiter plates ranged from 56 ng per well for *Streptococcus bovis* to 268 ng per well for *S. ruminantium* GA31. Culture of the cells in RF or DF medium prior to coating them onto the polystyrene did not appear to affect binding of cell protein. Overall adsorbed cell protein concentration was 130.1 ± 14.5 ng of protein per well, and the average coefficient of variation within bacterial strain was 15.8%. It was decided that the variation among bacterial species and strains would not unduly influence ELISA studies.

TABLE 1. Antibody and cell suspension titers for homologous reactions between egg yolk antibodies and selenomonad strains cultured in two media

Strain	Medium ^a	Week ^b	Antibody ^c			Cell suspension ^d	
			A ₄₉₀ ^e	Dilution ⁻¹	Normalized activity ^f	A ₄₉₀	Dilution ⁻¹
PC18	RF	3	1.10 ± 0.05 ^g	50	7.64	1.01 ± 0.11	50
		7	1.07 ± 0.04	50	1.07	1.29 ± 0.02	50
	DF	3	0.39 ± 0.02	50	2.45	0.63 ± 0.11	6.25
		7	0.59 ± 0.07	25	3.87	0.92 ± 0.01	6.25
GA192	DF	3	0.64 ± 0.01	100	8.23	0.50 ± 0.16	6.25
		7	1.12 ± 0.02	100	3.78	1.01 ± 0.02	50
HD ₄	DF	3	1.03 ± 0.04	200	27.60	1.23 ± 0.01	25
		7	1.03 ± 0.07	100	7.85	1.27 ± 0.02	50
D	DF	3	1.26 ± 0.03	3,200	564	1.04 ± 0.07	400
		7	1.14 ± 0.05	3,200	483	1.07 ± 0.07	1,600

^a See Materials and Methods for composition.^b Week that egg was collected post-initial immunization.^c The cell suspension concentration was kept constant at a reciprocal dilution of 100.^d The antibody concentration was kept constant at a reciprocal dilution of 100.^e A₄₉₀ represents homologous reaction between antibody and antigen grown in the same medium.^f A₄₉₀/mg of protein per ml of antibody preparation.^g SEM, Standard error of the mean.

Antibody characterization. Antibody preparations were characterized for their homologous titers and sensitivities (Table 1). Antibody and cell suspension dilutions listed are either the greatest dilution which gave an ELISA A₄₉₀ of 1.0 or, if below 1.0, the greatest dilution which gave the highest A₄₉₀. The average protein concentration across all antibody preparations examined in this study was 7.10 ± 0.87 mg/ml. The reactivity of the antibody preparations was normalized by dividing the A₄₉₀ of the indicated antibody dilution by the concentration of protein present in that dilution of antibody preparation. Data collected with PC18 indicated that the medium for culture of the immunizing antigen had little influence on normalized antibody activity, although antibody titer was greater for cells cultured in RF medium. The hen immunized with strain D gave the highest titers of all hens immunized. Absorbance per unit of antibody protein concentration was higher for the week 3 than the week 7 antibodies except for PC18 grown in DF medium. Antibody-specific activities were almost 100-fold higher for D and 3-fold higher for HD₄ (week 3) than for any other homologous combination.

Antibody sensitivity to bound cells was evaluated by determining the titer of the cell suspension. Except for PC18, week 7 antibodies raised against strains cultured in DF medium detected greater dilutions of cells than did the week 3 antibodies. The strain D antibody sensitivity was at least eight times greater than that of any of the other antibody preparations.

Cross-reactivity of selenomonad antibodies. In order to compare heterologous reactivities, a reciprocal dilution of 100 for both cell suspension and antibody preparations was chosen. In most cases an antibody dilution of 100 was close to or was the optimal titer for homologous reactivities (data not shown).

The cross-reactivities of selenomonad antibodies with other strains of *S. ruminantium* and other species and genera of ruminal and nonruminal bacteria were examined (Table 2). All cell suspensions coated onto titer plates originated from cultures grown in the complex RF medium since nutritional requirements were not known for some of the bacterial species. Preliminary experiments revealed that when selenomonad antibodies were reacted with selenomonad cells cultured in either RF or DF medium and

subsequently formalinized and washed, culture medium had no effect ($P > 0.10$) on the degree of antibody cross-reactivity. Week 3 antibodies made to four selenomonad strains cultured in DF medium (Table 1) were used. Week 7 antibodies were not used because these were no more specific than week 3 antibodies (data not shown). All of the homologous antibody-antigen combinations were more reactive ($P < 0.05$) than any of the heterologous combinations. Antibodies made to the four *S. ruminantium* strains readily differentiated the heterologous strains from the corresponding homologous cells by giving at least a threefold difference in cross-reactivity. The greatest cross-reactivity was among the *S. ruminantium* strains and with *S. putigena* and *Succinivibrio dextrinosolvens*. PC18 was a notable exception due to its uniformly low cross-reactivity with D, GA31, and all non-*S. ruminantium* organisms.

Quantification of strains. Quantification of strains GA192 and D in biculture cell suspensions was considered feasible because (i) similar amounts of whole-cell protein bound to well surfaces (95 to 107 ng per well) with 100-fold dilutions of both strains harvested from DF medium, (ii) these amounts of adsorbed protein resulted in nearly maximal ELISA absorbances compared with less dilute cell suspensions, (iii) the selected GA192 and D antibody dilutions (100- and 1,600-fold, respectively) resulted in high but submaximal ELISA absorbances, (iv) concentrations of secondary antibody with peroxidase conjugate and substrates were saturating because the final A₄₉₀ did not respond when the concentration of any of these components was increased, and (v) A₄₉₀ development was still linear for incubation times up to 60 min. For detection and quantification of cells, the optimal titer of antibody was taken to be the greatest dilution that still gave an A₄₉₀ above 1.0 but exhibited the lowest cross-reactivity. Cross-reactivities for the dilutions of GA192 and D antibodies with the D and GA192 cells used here were 7.8 and 2.1%, respectively.

The relationships of culture volume ratio with direct cell counts and ELISA A₄₉₀ are shown in Fig. 1 and 2, respectively. For most mixtures of the two strains, proportional changes in direct cell counts were observed. The notable exceptions were mixtures containing 70 to 90% GA192. Figure 2 reveals that A₄₉₀ changed proportionately with changes in culture volume ratio, except for mixtures con-

TABLE 2. Heterologous reactivity of week 3 polyclonal hen egg yolk antibodies to *S. ruminantium* strains, ruminal bacteria, and nonruminal bacteria grown in RF medium

Strain	Cross-reactivity (%) in ELISA of antibodies prepared against strain ^a :			
	PC18	HD ₄	GA192	D
<i>S. ruminantium</i>				
PC18	100.0 ± 2.3 ^g	26.0 ± 2.6 ^{i,j}	9.4 ± 1.9 ^b	18.1 ± 1.9 ^f
HD ₄	17.9 ± 3.2 ^f	100.0 ± 1.7 ^k	19.4 ± 1.9 ^{d,e}	25.6 ± 2.3 ^{h,i}
GA192	11.7 ± 3.2 ^{e,f}	14.2 ± 2.6 ^{d,e,f,g}	100.0 ± 1.9 ^h	14.8 ± 1.9 ^{e,f}
D	5.6 ± 2.6 ^d	19.0 ± 2.3 ^{f,g,h,i}	10.8 ± 1.9 ^b	100.0 ± 1.3 ^j
GA31	4.7 ± 3.2 ^{c,d,e}	16.6 ± 2.6 ^{e,f,g,h}	17.2 ± 3.2 ^{c,d,e}	7.5 ± 3.2 ^{b,c,d}
<i>S. putigena</i> 35185	2.7 ± 3.2 ^{b,c,d}	34.1 ± 2.6 ^j	26.8 ± 3.2 ^{e,f}	19.5 ± 3.2 ^{f,g}
<i>M. elsdenii</i> T81	0 ± 3.2 ^b	12.8 ± 2.3 ^{d,e,f}	20.6 ± 3.2 ^{d,e}	11.3 ± 2.3 ^{d,e,f}
<i>Salmonella typhimurium</i> TA831	0 ± 3.2 ^b	5.9 ± 2.6 ^{b,c}	14.4 ± 3.2 ^{b,c,d}	9.7 ± 3.2 ^{c,d,e}
<i>Succinivibrio dextrinosolvens</i>				
22B	0 ± 3.2 ^b	22.3 ± 2.3 ^{g,h,i}	33.2 ± 3.2 ^{f,g}	18.5 ± 2.3 ^{e,f,g}
24	0 ± 3.2 ^b	21.6 ± 2.3 ^{g,h,i}	36.8 ± 3.2 ^{f,g}	18.6 ± 3.2 ^{e,f,g}
<i>Butyrivibrio fibrisolvens</i> D1	2.0 ± 3.2 ^{b,c}	9.8 ± 2.6 ^{c,d,e}	14.0 ± 3.2 ^{b,c,d}	34.8 ± 3.2 ⁱ
<i>Bacteroides ruminicola</i> B ₁ 4	0.3 ± 3.2 ^b	25.4 ± 2.6 ^{h,i,j}	13.5 ± 3.2 ^b	7.4 ± 3.2 ^{b,c,d}
<i>Streptococcus bovis</i> JB1	0 ± 3.2 ^b	2.2 ± 2.6 ^b	14.0 ± 3.2 ^{b,c,d}	4.3 ± 3.2 ^{b,c}
<i>L. plantarum</i> 14917	0.8 ± 3.2 ^b	8.0 ± 2.6 ^{c,d}	6.4 ± 3.2 ^b	2.4 ± 3.2 ^b

^a Results are expressed as the mean ± standard error of the mean ($n = 2$ to 4 averages of duplicate determinations). Means in the same column with different superscripts differ ($P < 0.05$).

taining 70 to 90% GA192. The relationships between direct cell counts and A_{490} implied by the results shown in Fig. 1 and 2 are shown in Fig. 3 and 4 for strains D and GA192, respectively. Logarithmic transformation of the direct cell counts was necessary to linearize the relationship. Without transformation, the curvilinear relationship was interpreted to reflect the saturable nature of cell binding to the polystyrene well surfaces. For both strains, direct cell counts accounted for at least 90% of the variation in A_{490} . These relationships were obtained for cell population sizes which were varied over the range of 1 logarithmic unit.

DISCUSSION

The distinguishing feature between the two subspecies of *S. ruminantium* reported earlier (2) and confirmed here is the

FIG. 1. Direct cell counts of two strains of *S. ruminantium* subsp. *ruminantium* in biculture suspensions.

ability of strains of *S. ruminantium* subsp. *lactilytica* to grow on lactate. In addition, both strains of *S. ruminantium* subsp. *ruminantium* and the *S. ruminantium* subsp. *lactilytica* strain GA31 were able to ferment trehalose.

Our previous efforts which addressed the immunological identification of ruminal bacteria (16) have been confirmed and extended here. We were able to confirm our previous findings that 100-fold dilutions of cell suspensions and antibody preparations allowed for effective comparisons among heterologous reactivities of antibodies made to various ruminal selenomonad strains. In addition, immunological differences among bacterial strains were not due to differences in cell adsorption onto the titer plates. GA31 had the highest

FIG. 2. ELISA reactivity (A_{490}) of antibody preparations against two strains of *S. ruminantium* subsp. *ruminantium* with mixtures of cell suspensions of the two strains.

FIG. 3. Quantification of *S. ruminantium* D when present in a mixed-cell suspension composed of *S. ruminantium* D and GA192. ELISA A_{490} (y) = $-8.9 + 1.3x$ where $x = \log_{10}$ direct cell counts of strain D ($R^2 = 0.92$).

degree of cell adherence to the microtiter plate wells, but in no case was it the most cross-reactive strain with *S. ruminantium* antibodies (Table 2).

To further characterize the specificity of egg yolk polyclonal antibodies, their cross-reactivities with a contrasting set of ruminal and nonruminal bacteria were tested. A surprise was the unprecedented close relatedness of the two *Succinivibrio* strains with the ruminal selenomonads. Although these organisms are grouped in the same family (5), the relatedness of selenomonads and succinivibrios has not been investigated with other techniques. The cross-reactivities of week 3 strain D antibody with *Bacteroides rumini-*

FIG. 4. Quantification of *S. ruminantium* GA192 when present in a mixed-cell suspension composed of *S. ruminantium* GA192 and D. ELISA A_{490} (y) = $-3.8 + 0.7x$ where $x = \log_{10}$ direct cell counts of strain GA192 ($R^2 = 0.90$).

cola B₁₄ and *Streptococcus bovis* JB1 were lower here than those in our previous work (16), but in both studies the strain D antibody was twofold more reactive with B₁₄ than in JB1 cells.

It has been suggested on the basis of 16S rRNA data that *S. ruminantium* and *M. elsdenii* actually group more closely with the gram-positive eubacteria of the "Clostridium subdivision" than with gram-negative eubacteria (21). Although the investigators documented several lines of evidence to support this hypothesis, *M. elsdenii* and the selenomonads did not seem to be immunochemically similar here. One concern was that the presence of flagella might skew the data in favor of reactivity, but the nonruminal *Salmonella typhimurium* was less reactive than many of the nonflagellated organisms.

Overall, it can be concluded that these polyclonal selenomonad antibodies have sufficient specificity to distinguish between selenomonads and nonselenomonads and among species within the two groups. Mixing known proportions of *S. ruminantium* strains and counting each of the strains in these mixtures allowed us to conclude that the ELISA values matched the corresponding changes in direct cell counts. The ELISA was capable of detecting population shifts to 1 log unit, which exceeds the sensitivity of current nonimmunochemical enumeration techniques. Application of this methodology to a ruminal sample will probably require even more antibody specificity than has been shown here. Enhanced antibody specificity could potentially be gained by shortening the reaction time, prior cross-adsorption with heterologous strains to remove antibodies that react with shared epitopes, and purification of the immunogen to create a more specific immune response.

Several applications of strain-specific egg yolk polyclonal antibodies in ruminal studies appear plausible. It appears feasible to generate a battery of very specific egg yolk polyclonal antibodies against some of the better characterized ruminal bacterial cultures and to use these antibodies as diagnostic probes in a myriad of ecological investigations as diverse as identifying colonies on a plate or immunochemical marking of cells in situ. An important issue in this scenario of using strain-specific antibodies is the relevancy of bacterial strains maintained in culture collections versus freshly isolated ruminal strains. It remains to be determined whether a D- or HD₄-like strain can be isolated from the rumen and exhibit sufficient reactivity to be identified as such or whether immunological specificity is unique to the captive ruminal isolate. Nevertheless, the specificity of the antibodies described here makes them well suited for rapid detection and quantification of a genetically altered bacterial species in the rumen (18). Since stability of an identifiable epitope is required (18), fusion of the desired DNA construct with an immunologically significant cell surface component would enhance the generation of specific antibodies, facilitate optimization of the ELISA, and provide a direct assay for expression of the genetic alteration in the intact cell.

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