VALIDATION OF AN ELISA USING POLYCLONAL CHICKEN ANTIBODIES TO DETECT AND QUANTIFY TWO STRAINS OF *SELENOMONAS RUMINANTIUM* IN BICULTURE SUSPENSIONS

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Introduction

Selenomonas ruminantium is a predominant rumen bacterial species. Its importance has been assessed via direct counts in rumen fluid, and by isolation and presumptive identification. The latter technique requires several days for growth of colonies and then further nutritional and physiological characterization. Neither technique allows for immediate identification nor quantification beyond the species level, and certainly not of individual strains. Ricke et al. (1988) found that polyclonal antibodies harvested from chicken egg yolks and used in an enzyme-linked immunosorbent assay (ELISA) were specifically reactive with the immunizing species. The objectives of these experiments were: (1) to determine the degree of species and strain specificity of polyclonal chicken antibodies prepared with five selenomonad strains as antigens, and (2) to quantify the population size of each of two selenomonad strains in biculture cell suspensions.

Materials and Methods

Strains D, GA192, PC18, HD4 and HD1 of *Selenomonas ruminantium* were cultured in a defined medium (Ricke et al., 1988), harvested near late log phase, washed with phosphate-buffered saline and fixed with 8% formal saline. Each strain was injected twice into one Leghorn laying hen. For the determination of antibody specificity for strains and species, antibodies were harvested by polyethylene glycol precipitation (Ricke et al., 1988) from a pre-immunization egg yolk (Day 0) and from yolks of eggs laid 20-26 days after initial immunization. A variety of non-rumen and rumen bacterial species (table 1) were cultured in a rumen fluid-containing medium and utilized in ELISAs as indicated above. Polystyrene microtitration plate wells were filled with 100 µl of 100-fold dilution of whole cell suspension (0.36-1.66 µg protein) which was allowed to adsorb overnight. Antibody preparations diluted 100-fold (100 µl containing 0.7 mg protein) were added and allowed to react for 1 h. ELISAs were conducted with equipment, chemicals and procedures used by Ricke et al. (1988). Bacterial cell protein bound to surfaces of wells was assayed (Sorensen and Brodbeck, 1986).

TABLE 1. HETEROLOGOUS REACTIVITY OF POLYCLONAL EGG YOLK ANTIBODIES OF BOVINE *SELENOMONAS RUMINANTIUM* STRAINS

Cells in ELISA	Antibodies prepared against strain			
	PC18	HD4	GA192	D
	Cross-reactivity (%)			
S. ruminantium				
PC18	–[a]	21±2[b]	10±4	29±3
HD4	23±1	–	15±0.5	32±4
GA31	5±2	17±4	17±1	8±3
GA192	10±0.3	13±5		17±2
D	14±5	18±4	20±8	–
S. sputigena 35185	ND[c]	34±3	27±4	20±2
Succinivibrio dextrinosolvens 22B	ND	22±3	33±2	19±2

[a]Homologous reaction.
[b]SEM (n=2 to 4).
[c]Not detectable.

Quantification of population sizes in a biculture cell suspension was performed with strains D and GA192. These strains were chosen because they are members of the same subspecies, they are morphologically distinguishable, and antibody titers against these strains were high with relatively low cross-reactivity. Direct microscopic counts prior to mixing were 8.05×10^7 cells/ml for D and 2.75×10^7 cells/ml for GA192. Eleven mixtures of the two strains were prepared beginning with 100:0 (D:GA192), followed by substitutions

of 10 units. Aliquots of 100 µl of each mixture were plated into duplicate wells. Antibodies to strain D were obtained from a day 26 egg and diluted 1600-fold while GA192 antibodies from a day 41 egg were diluted 100-fold.

Results

S. ruminantium strain HD1 cells were more reactive with antibodies raised against strain HD4 than were strain HD4 cells. Further evaluation of strains HD1 and HD4 revealed that HD1 could not be distinguished from HD4 based upon carbohydrate utilization and growth rates on lactate, glycerol and trehalose; identity and proportions of organic acids produced from glucose; and cell morphology. Subcultures of HD1 from three reputable culture collections gave these same physiological results.

Cross reactivities of egg yolk antibodies raised against four selenomonad strains are given in table 1. Cross-reactivity of selenomonad antibodies was generally greater with *S. ruminantium* strains than with other rumen species (*Bacteroides ruminocola* B_14, *Butyrivibrio fibrisolvens* D1, *Streptococcus bovis* JB1, and *Megasphaera elsdenii* T81) and the non-rumen species *Salmonella typhimurium* TA831 and *Lactobacillus plantarum* 14917). Notable exceptions were high cross-reactivities of HD4, GA192 and D antibodies with *S. sputigena*, the type species for the genus, and *Succinivibrio dextrinosolvens*. Cell protein bound in the wells ranged from 57-268 ng/well for selenomonad strains and the other species.

Quantification of strains GA192 and D in biculture cell suspensions was considered feasible because (1) similar amounts of cell protein bound to well surfaces (97-107 ng/well) with 100-fold dilutions of both strains, (2) these amounts of bound protein resulted in nearly maximal ELISA absorbances compared to less dilute cell suspensions, (3) the selected GA192 and D antibody titers resulted in high but submaximal ELISA absorbances, (4) secondary antibodies with peroxidase conjugate and enzyme substrates were saturating, and (5) incubation times of 60 min for color development were still linearly responsive. Cross-reactivities of GA192 and D antibodies with D and GA 192 cells were 7.8 and 2.1%, respectively. The relationships for reciprocal dilution of cells (x) added to wells and ELISA absorbance at 490 nm (y) were $y = 18.84\ x^{0.555}$ ($R^2 = 0.97$) for strain D, and $y = 12.10\ x^{-0.480}$ ($R^2 = 0.93$) for strain GA192. Similar relationships between reciprocal dilution of cells (x) and direct cell counts (y) added to wells were $y = 1.67 \cdot 10^9 \cdot x^{-0.72}$ ($R^2 = 0.91$) for strain D, and $y = 5.94 \cdot 10^9 \cdot x^{-1.14}$ ($R^2 = 0.93$) for strain GA192.

Discussion

Polyclonal egg yolk antibodies were useful for discerning the HD1 cultures currently available are not able to be distinguished from the HD4 strain, as originally described (Bryant, 1956), and are probably mislabeled HD4 cultures. This antibody source is humanely obtained, inexpensively prepared and holds promise for quantifying cell population responses of known bacterial strains grown in defined bi- and multicultures.

(Key Words: ELISA, *Selenomonas ruminantium*, Antibodies)

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