APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1997, p. 4069–4074 Vol. 63, No. 10
0099-2240/97/$04.00+0

Hydroxyapatite Adherence as a Means To Concentrate Bacteria

ELAINE D. BERRY AND GREGORY R. SIRAGUSA*

Roman L. Hruska U.S. Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture, Clay Center, Nebraska 68933-0166

Received 7 April 1997/Accepted 1 July 1997

Adherence to hydroxyapatite (HA) was examined as a method to concentrate bacteria from foods. Using HA at a level of 10% and suspensions of an *Escherichia coli* strain containing 10^9, 10^6, and 10^3 cells per ml, kinetic studies revealed that maximum adherence was attained within 5 min for all cell concentrations and that comparable log reductions (1.0 to 1.5) of cells in suspension were seen regardless of initial cell concentration. Eleven species of spoilage and pathogenic bacteria were found to adhere to HA, with seven species adhering at proportions of greater than 95%. Fluorescent viability staining revealed that cells bound to HA remained viable. There was greater than 92% adherence of indigenous bacteria to HA from three of five 1:10 dilutions of ground beef, indicating promise for the use of HA for concentrating bacteria from meat and other food samples.

There is a great need for rapid microbial testing in the food industry. This need has long been apparent in situations where results are needed for approval or rejection of raw materials or for the release of a held batch of product. In addition, the need for rapid, on-line testing has become greater with the increasing implementation of hazard analysis and critical control point programs by the food industry (2). Advances in rapid microbiological methods such as nucleic acid probe hybridization or immunological assays have dramatically shortened the time required for detection of bacterial pathogens in meats and other foods. However, these methods typically require concentrations of the target microorganism of ca. 10^4 to 10^6 cells per ml or more (5, 18, 19). As bacterial pathogens usually occur in low numbers in foods, an enrichment incubation of at least several hours to a day or more is required prior to the application of a rapid detection assay or a selective culture method. While PCR-based assays have the potential to overcome this limitation, most PCR procedures for the detection of food-borne pathogens require a selective enrichment step prior to amplification, which serves to dilute PCR-inhibitory food components and to concentrate bacteria which may be present at concentrations of less than 1 cell per ml or g of food (18). The need for rapid, on-line microbial testing calls for the development of methods to circumvent this incubation time, via enrichment by noncultural concentration of the microorganisms from the food. In addition to shortening the time required for detection results, efficient methods for bacterial concentration from foods would allow for the collection of larger and more representative samples, thereby increasing the probability of detection.

Methods for separating and concentrating microorganisms from foods have been reviewed by Payne and Kroll (15). The more commonly used physical methods for separation and concentration of bacteria are centrifugation and filtration, but the application of these methods to food products may be limited due to interference from food particulates or chemical properties of food components. Other physical methods that have been attempted take advantage of cell surface characteristics; such methods include the use of anion- and cation-exchange resins and two-phase extraction systems (15).

Hydroxyapatite (HA) has been used extensively as a matrix for the purification and fractionation of an array of biochemical substances, including enzymes, nucleic acids, hormones, and viruses (6). Research describing applications of HA to whole bacterial cells is focused almost exclusively in the area of oral microbiology. A large proportion of the inorganic portion of dental enamel consists of HA, and HA has been useful as a model for the study of dental plaque formation (7–10, 12, 20, 21). Many of these works describe HA adsorption of high cell numbers of different species of bacteria, which prompted our attention. In this work, bacterial adherence to HA was examined for use in concentration of bacteria from suspensions. Our studies (i) examined the HA adherence characteristics of several different species of food-borne spoilage and pathogenic bacteria and (ii) investigated factors affecting additional cell adherence to HA. An additional objective was the demonstration of the potential use of HA to remove and concentrate indigenous bacteria from ground beef, feces, and beef carcass sample suspensions.

Bacterial strains and culture conditions. The bacterial strains used in this work are listed in Table 1. Experimental cultures were grown overnight at 37°C in Trypticase soy broth (BBL, Cockeysville, Md.) supplemented with 0.5% yeast extract, except for *Carnobacterium divergens*, which was grown overnight at 30°C, and *Pseudomonas fluorescens* and *Brochothrix thermosphacta*, which were grown 24 and 48 h, respectively, at 25°C. For adherence experiments, cells were collected by centrifugation (8,160 × *g*, 5 min), washed twice in filter-sterilized adsorption buffer (5 mM KCl, 1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 1 mM K_2HPO_4 [pH 7.2]) (9), and then diluted in the same buffer to the desired inoculum level.

Bacteria were enumerated on tryptic soy agar containing 0.5% yeast extract (Difco, Detroit, Mich.). Bacteria were serially diluted as necessary in 2% buffered peptone water (BPW; BBL) and plated in duplicate by spread plating or by using a spiral plater (model D; Spiral Biotech, Bethesda, Md.). According to the species being examined, the plates were incubated for 24 or 48 h at the temperatures noted above prior to colony enumeration. Bacterial populations were converted to $\log_{10}$ CFU/milliliter values prior to statistical analyses. Analysis of variance and the Tukey-Kramer multiple comparisons test, or Student's *t* test, were performed on transformed bacterial population or percent adherence data, using the InStat2 ver-

* Corresponding author. Mailing address: U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Spur 18D, Clay Center, NE 68933-0166. Phone: (402) 762-4227. Fax: (402) 762-4149. E-mail: siragusa@marcvm.marc.usda.gov.

TABLE 1. Adherence of bacteria to HA

Bacterial species	Cells available for adherence (CFU/ml in control [no HA])[a]	% Adherence (SD)[a,b]
Escherichia coli ATCC 25922	1.45×10^5	98.6 (0.5)A
Brochothrix thermosphacta ATCC 11509	1.20×10^6	98.8 (1.6)A
Carnobacterium divergens ATCC 35677	5.49×10^4	96.7 (2.9)A
Enterococcus faecalis ATCC 19433	1.35×10^5	96.6 (1.6)A
E. coli ATCC 43895 (serotype O157:H7)	1.38×10^5	45.8 (9.7)B
E. coli ATCC 35150 (serotype O157:H7)	3.06×10^5	54.0 (11.0)B
Listeria monocytogenes Scott A	5.58×10^4	98.9 (0.4)A
Pseudomonas fluorescens ATCC 13525	3.57×10^5	93.5 (0.6)A
Salmonella typhimurium ATCC 14028	1.12×10^5	87.5 (5.3)A
Staphylococcus aureus ATCC 25923	3.53×10^4	99.6 (0.3)A
Yersinia enterocolitica ATCC 23715	1.24×10^5	99.0 (0.2)A

[a] Mean of two independent trials in which experiments were performed in duplicate.
[b] Values followed by the same letter are not significantly different ($P \leq 0.001$).

sion 2.0 statistical analysis package (GraphPad Software, San Diego, Calif.).

HA preparation. HA (H-0252; Sigma Chemical Co., St. Louis, Mo.) was washed three times in 5 volumes of sterile deionized, distilled H_2O. Appropriate volumes of HA were then measured into glass test tubes and washed three times with 10 volumes of adsorption buffer. The HA was then resuspended in adsorption buffer, sterilized by autoclaving, and stored at 4°C. Before use in adherence assays, the HA volumes were allowed to come to room temperature, and the overlying buffer was removed. Except where noted, adherence of bacterial cells to HA was examined by using HA at a level of 10% (vol/vol).

HA adherence kinetics of different cell concentrations. Examination of the rate of bacterial adherence to HA utilized a range of cell concentrations to determine if there were differences in adherence rates from suspensions containing dilute or concentrated cells. *E. coli* ATCC 25922 was suspended to levels of 10^9, 10^6, and 10^3 cells per ml in adsorption buffer, which were designated high, intermediate, and low levels of cells, respectively. Appropriate volumes of the cell suspensions were added to measured volumes of HA, such that HA was 10% of the total volume. The tubes were inverted to mix the contents, laid horizontally on a shaker platform, and mixed by gentle rocking to keep contents suspended, for 2 h at room temperature. At 15-min intervals, the tubes were again inverted to mix contents, samples were removed to sterile microcentrifuge tubes, and HA was allowed to settle for 5 min. Cells in the resulting supernatants were enumerated. A comparison of cell populations in the initial suspensions and in the controls (without HA) showed that *E. coli* remained viable over the 2-h incubation. For high, intermediate, and low cell levels, adherence to HA over the 120-min period resulted in mean log reductions ($n = 4$) of 1.44, 0.91, and 1.76 (corresponding to 96.2, 84.4, and 95.9% adherence), respectively. Essentially all adherence of *E. coli* to HA took place within 15 min, with little or no additional adherence taking place over the continuance of the 120-min incubation, even for the lower concentrations of cells. The experiment was subsequently repeated, monitoring cell populations remaining in suspension over a 15-min period. Results showed that at all three cell levels, the majority of adherence to HA took place within 1 min. Therefore, subsequent HA adherence experiments were conducted with a 5-min HA extraction.

Adherence of other bacterial species to HA. The HA adherence characteristics of 11 species of spoilage and pathogenic bacteria significant in meats and other foods were investigated. Cells were diluted in adsorption buffer to a target level of ca. 10^5 cells per ml, and 4.5-ml volumes were added to 0.5-ml volumes of HA. The adherence experiments were conducted as described above except that the tube contents were extracted by mixing for only 5 min prior to HA settling. Percent adherence to HA was calculated by difference of cell numbers remaining in supernatants and cell numbers in controls that did not contain HA.

Results are shown in Table 1. For eight species, more than 90% of available cells adhered to HA, with the majority exhibiting greater than 95% adherence. Of all bacteria examined, only two strains of *E. coli* serotype O157:H7 did not adhere substantially to HA (ca. 50% of available cells adhering). It will be interesting to discover if this is a common feature among *E. coli* O157:H7 and to discover if HA extraction conditions can be manipulated to effect greater adherence of this important pathogen. This experiment demonstrates that a wide range of species will adhere to HA and subsequently be concentrated; thus, HA may be useful for applications requiring the concentration of either generic or specific bacteria from foods.

Viability of cells bound to HA. A cell suspension containing 10^6 *E. coli* 25922 cells per ml in adsorption buffer was extracted with 10% HA. Following HA settling, the supernatant was removed and 0.1 ml of the HA fraction was removed to 0.9 ml of sterile, filtered (0.2-μm-pore-size filter) water. This volume was stained by using LIVE/DEAD *Bac*Light viability fluorescent staining (Molecular Probes, Eugene, Oreg.), and portions were examined in a fluorescence microscope (Carl Zeiss, Inc., Thornwood, N.Y.). Cells bound to HA fluoresced green, indicating viability (Fig. 1). In addition, it was observed that more than one cell may be associated with a HA crystal, providing visual evidence of multiple cell binding to HA.

Factors affecting cell adherence to HA. Dental plaque models primarily have studied cell adherence to saliva-coated HA. Saliva-coated HA approximates the natural conditions of the acquired salivary pellicle, a membranous film composed of a variety of salivary proteins and other constituents that covers the teeth (reviewed by Liljemark and Bloomquist [13]). Bacterial cell adsorption to the acquired pellicle and saliva-coated HA may be due to both nonspecific bonding and specific interactions, including hydrophobic, electrostatic, and lectin-like adhesin-receptor interactions (3, 10, 11, 14). Bacterial cell adherence to bare HA, which was studied in this work, is mediated by nonspecific van der Waals and electrostatic attractions (10, 14). The adsorption of bacteria, which possess a net negative charge, to bare HA appears to be similar to the adsorption of negatively charged proteins, which is due to the electrostatic attraction between the negatively charged molecule and the positively charged calcium atoms of the HA (4, 16). In the kinetic studies reported here, we noted that cell adherence to HA, in terms of log reduction values or percent adherence from suspension, was similar for all three cell concentrations examined. The adherence of 96.2% of an initial level of 10^9 *E. coli* per ml indicated that HA has a high capacity for cell binding; therefore, factors other than available HA surface area were implicated in limiting additional cell adherence. Thus, factors affecting further cell adherence to HA were investigated.

As an initial experiment, the effects of reextraction of cell suspensions and HA fractions on additional cell adherence were examined. *E. coli* ATCC 25922 was suspended to a level of 10^9 cells per ml in adsorption buffer, and 9.0-ml volumes were added to 1.0-ml volumes of HA. Following HA extrac-

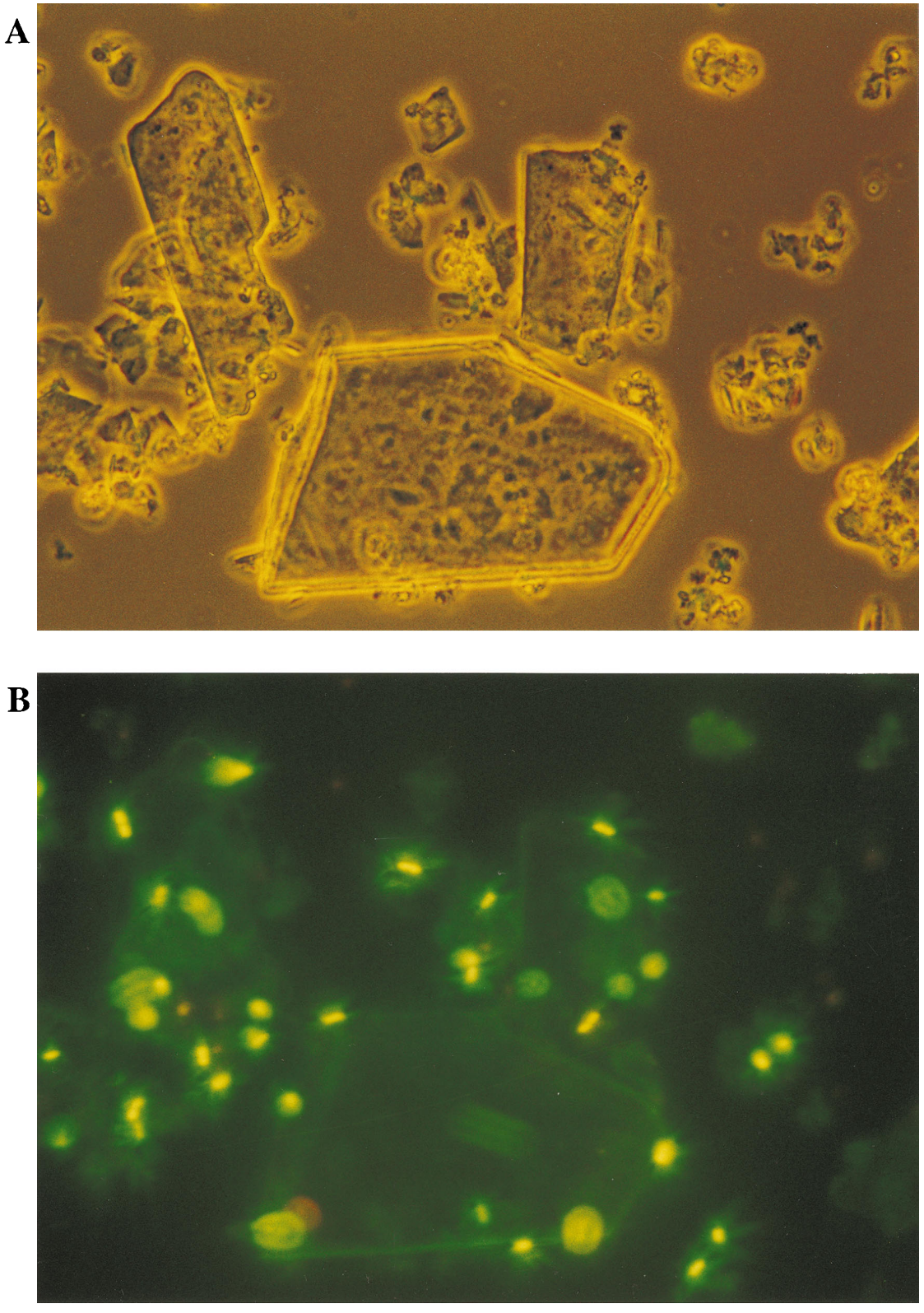

FIG. 1. Fluorescent viability staining of *E. coli* ATCC 25922 cells adhered to HA. Following HA extraction of a suspension of *E. coli* in HA adsorption buffer, the HA fraction was stained with a two-color fluorescence viability stain. *E. coli* fluoresced green, indicating that the cells remain viable when adhered to HA. (A) Bright field; magnification, ×1,000. (B) Fluorescence micrograph of the same field of view showing multiple viable cells bound to the individual HA crystals.

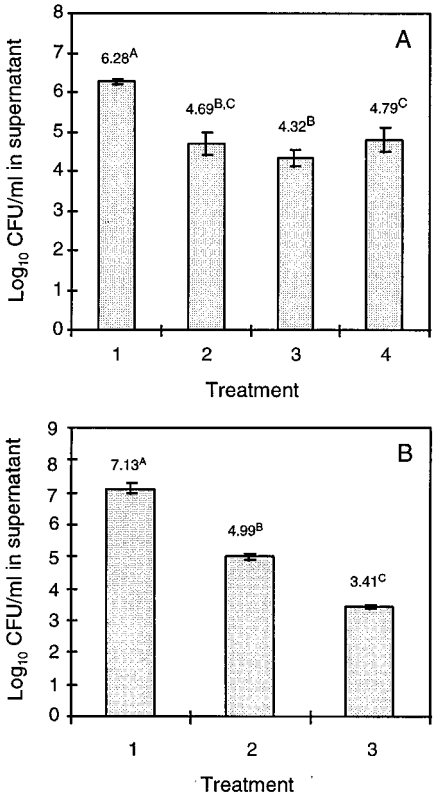

FIG. 2. Effect of reextraction of supernatant, HA and cell fractions on additional adherence of *E. coli* ATCC 25922 to HA. (A) Treatments: 1, cell concentration in original suspension; 2, cell concentration in supernatant following first extraction of cell suspension on HA; 3, cell concentration in supernatant following second extraction of cell suspension (from treatment 2) on fresh HA; 4, cell concentration in supernatant following extraction of fresh cell suspension (treatment 1) on once-extracted HA (from treatment 2). Data are means of two independent trials with all experiments performed in triplicate ($n = 6$). Error bars indicate standard deviations. Values followed by the same letter are not significantly different ($P \leq 0.05$). (B) Effect of fresh adsorption buffer on additional adherence of extracted *E. coli* ATCC 25922 to HA. Treatments: 1, cell concentration in original suspension; 2, cell concentration in supernatant following first extraction with HA; 3, cell concentration in supernatant following centrifugation collection of cells from supernatant (from treatment 2), resuspension in fresh adsorption buffer, and second extraction on fresh HA. Data are means of two independent trials with all experiments performed in duplicate ($n = 4$). Error bars indicate standard deviations. Values followed by the same letter are not significantly different ($P \leq 0.001$).

tion, the supernatants were removed to sterile test tubes, and the HA fractions were reserved. The supernatants were sampled for enumeration, and then 4.5-ml volumes were reextracted with 0.5 ml of fresh HA. To the reserved HA fractions, 9.0-ml volumes of the original cell suspension were added and extracted. Following these second extractions, cell numbers remaining in the supernatants were determined. In an additional experiment, the effect of fresh adsorption buffer on HA adherence of bacterial cells remaining in extracted supernatant was determined. Nine milliliters of *E. coli* ATCC 25922 (10^7 cells/ml) was extracted with 1.0 ml of HA. Following sampling for enumeration, the cells in the remaining supernatant were collected by centrifugation (1,200 $\times$ *g*, 15 min) and resuspended to the original volume in fresh adsorption buffer. Volumes of 4.5 ml of the resuspended cells were then reextracted on 0.5 ml of fresh HA, and cells remaining in the supernatant were enumerated.

The results of these reextraction experiments are shown in Fig. 2. In the first experiment (Fig. 2A), HA extraction of the original cell suspension resulted in a log reduction of 1.59 of cells in suspension, consistent with previous data. The reextraction of this used cell suspension with fresh HA resulted in a statistically insignificant ($P \leq 0.05$) additional log reduction of 0.37. However, when a fresh volume of the original cell suspension was extracted with the first, once-used HA fraction, there was a log reduction of 1.49. The $\log_{10}$ CFU/milliliter value of the remaining cells following this second extraction with HA (4.79) was not significantly different ($P > 0.05$) from that following the first extraction on the same HA fraction (4.69). The additional experiment was conducted to determine if the cell capacity limitation was due to changes in the buffer or changes in cells resulting from HA extraction. The effects of fresh adsorption buffer on additional cell adherence to HA are shown in Fig. 2B. When cells remaining in the supernatant following the first extraction were collected, resuspended in fresh buffer, and extracted again with HA, there was an additional log reduction of 1.58 of cells in suspension. The results of these two experiments indicate that changes in the adsorption buffer were a factor in limiting cell adherence to HA.

It has been reported that the ionic strength of the suspending buffer may affect bacterial adherence to bare HA (1, 21). In particular, calcium ions may be important in bacterial cell adherence to bare HA. The adsorption to bare HA of an avirulent *Actinomyces viscosus* was significantly enhanced by increases of calcium ions in the adsorption buffer from 0 to 5 mM, although the adsorption of its virulent counterpart was slightly inhibited (21). Adsorption to HA of *Streptococcus mutans* was increased by the inclusion of 0.5 M $CaCl_2$ (16). In addition to examining the effects of calcium, Wheeler et al. (21) studied the influence of pH, other cations, and detergents on adsorption of *A. viscosus* to HA and saliva-treated HA and found that environmental conditions of the adsorption buffer can exert significant effects on adsorption. In this study, we examined the effects of divalent cations and pH on cell adherence to HA.

E. coli ATCC 25922 was suspended to a level of 10^5 cells per ml in adsorption buffer containing EDTA or EGTA at various concentrations from 0 to 10 mM, $CaCl_2$ at concentrations from 0 to 5 mM, or $MgCl_2$ at concentrations from 0 to 0.5 mM. Cell suspensions were extracted with 10% HA that had been prepared in adsorption buffer of the same composition. Chelation of calcium or magnesium by the addition of EDTA to the adsorption buffer resulted in a statistically significant decrease in cell adherence to HA compared to buffer containing no EDTA ($P \leq 0.05$; data not shown); however, the increase in the $\log_{10}$ CFU/milliliter values of cells remaining in the superantant upon increasing the EDTA concentration was not significant ($P > 0.05$), and there was still substantial cell adherence to HA (91.8%) even in buffer containing 10 mM EDTA. The presence of EGTA in the adsorption buffer did not affect *E. coli* adherence to HA. The addition of 1 mM $CaCl_2$ to adsorption buffer with no $CaCl_2$ increased the log reduction from 1.19 to 1.58 from a suspension with an initial level of 5.66 $\log_{10}$ CFU/ml; however, the additions of increasing concentrations of $CaCl_2$ up to 5 mM and of $MgCl_2$ up to 0.5 mM in the buffer did not further increase cell adherence. In an additional experiment, the concentration of $MgCl_2$ was increased further to 1 and 2 mM, and no effect on cell adherence was observed. Similarly, changes in the pH of the adsorption buffer from pH 4.5 to 8.0 had no impact on the HA adherence of *E. coli* ATCC 25922. Clearly, more investigation will be required to determine buffer conditions that will promote bacterial cell adherence and result in further bacteria concentration.

The effect of HA concentration on removal of cells from

suspension was also examined. The use of HA at a level of 20% in the extractions resulted in a log reduction of 2.66 from the initial level of 6.08 $\log_{10}$ CFU/ml, corresponding to adherence of 99.8%. When the HA concentration in the extraction was reduced or increased, there was a concomitant reduction or increase in adherence of *E. coli*, with log reduction values ranging from 1.21 with 2.5% HA to 2.66 with 20% HA from an initial level of 6.08 $\log_{10}$ CFU/ml. While these log reduction values were significantly different ($P > 0.05$), the values seen with the lower HA concentrations (for example, 93.8% adherence with 2.5% HA) nevertheless represent substantial adherence. This observation implies that optimization of buffer conditions to enhance further cell adherence to HA should allow the use of lower concentrations of HA, an obvious advantage to further concentration of bacteria.

HA adherence of indigenous bacteria from suspensions of ground beef, beef carcass surface samples, and bovine feces. Five packages of fresh 80%/20% (lean/fat) ground beef were purchased from four different local retail grocery stores. Twenty-five gram samples of the ground beef were measured into filtered stomacher bags, 225 ml of adsorption buffer was added, and the samples were stomached for 2 min (Stomacher 400; Tekmar, Inc., Cincinnati, Ohio). These ground beef suspensions filtered 1:10 were further diluted to 1:100 and 1:1,000 in adsorption buffer. Duplicate suspensions were extracted with 10% HA. The initial ground beef suspensions and the supernatants remaining following extraction were sampled for bacterial enumeration.

Prerigor lean beef carcass surface tissue (cutaneous trunci) from six carcasses was collected immediately after slaughter at a local cow/bull processing facility and transported to the laboratory at the Roman L. Hruska U.S. Meat Animal Research Center. A 25-cm^2 sample was excised from each tissue piece and placed in a filtered stomacher bag, 25 ml of 2% BPW containing 0.1% Tween 20 (BPW-T) was added, and the contents were stomached for 2 min. Stomachates were extracted with HA, and bacteria in the initial stomachates and resulting supernatants were enumerated.

Five chilled beef carcasses were sponge sampled by the method of Siragusa et al. (17) at 24 h postslaughter at a local cow/bull processing facility. Samples were a composite of three 100-cm^2 sponged areas from the brisket, rump, and shoulder of the carcass, using BPW-T as the sponge solution. Following transport to the laboratory, the sponges and solutions were aseptically transferred to sterile filtered stomacher bags and stomached for 2 min. Duplicate volumes of the filtered stomachates were extracted with 10% HA.

Fresh bovine feces were collected from five individual cows fed a corn-silage ration. Ten grams of feces was measured into sterile filtered stomacher bags, 90 ml of adsorption buffer was added, and contents were stomached for 2 min. The 1:10 suspensions were further serially diluted to 1:100 and 1:1,000 in adsorption buffer. Duplicate volumes of each feces dilution were extracted with HA, and bacteria in the initial dilutions and HA supernatants were enumerated.

The results of HA adherence of indigenous microorganisms from ground beef, bovine feces, and beef carcass surface sample suspensions (Table 2) demonstrate the potential for HA in the application of concentrating bacteria from some sample types. Percent adherence values obtained for the 1:100 and 1:1,000 suspensions of both ground beef and feces were typically greater and less variable than those obtained for the 1:10 suspensions. These results are indicative of interference from particulates or other interfering food or fecal components in the 1:10 suspensions, whose effects were eliminated with further dilution. Percent adherence values of the 1:100 and 1:1,000 ground beef samples were not significantly different; thus, there was no realized increase in percent adherence of bacteria due to further dilution of the ground beef to 1:1,000. Although there was greater variation in percent adherence of bacteria in the 1:10 ground beef samples, in three of the five samples, more than 92% of the bacteria adhered. Similar results were seen with feces suspensions. Individual sample adherence values obtained for the 1:100 and 1:1,000 samples were typically greater than 90%, and from four of the five 1:1,000 feces suspensions, greater than 99.0% adherence was obtained. However, bacterial adherence values from the 1:10 dilutions of feces were much lower and more variable, ranging from 11.6 to 54.3%.

Adherence of bacteria in stomachates of excised bovine carcass surface tissue were low and highly variable, ranging widely from 0 to 46.3%. Percent adherence values of bacteria recovered in carcass sponge samples were also typically lower than those observed with ground beef or diluted feces suspensions.

TABLE 2. HA adherence of indigenous bacteria from ground beef, bovine feces, and beef carcass surface sample suspensions

Sample type	Sample no.	Dilution	Initial CFU/ml[a]	% Adherence (SD)[a,b]
Ground beef	1	1:10	2.66×10^6	92.3 (3.3)A
	2		1.60×10^5	72.5 (2.3)A,B
	3		2.27×10^5	29.8 (42.1)B
	4		2.66×10^5	92.5 (1.9)A
	5		2.31×10^5	94.3 (0.6)A
	1	1:100	2.41×10^5	97.7 (0.1)A
	2		1.80×10^4	95.1 (0.2)A
	3		5.34×10^4	93.8 (0.4)A
	4		4.17×10^4	98.8 (0.4)A
	5		2.13×10^4	99.5 (0.1)A
	1	1:1,000	2.62×10^4	99.2 (0.2)A
	2		1.74×10^3	94.7 (0.1)A
	3		5.41×10^3	94.9 (0.9)A
	4		4.48×10^3	98.0 (0.6)A
	5		2.95×10^3	99.5 (0.2)A
Excised bovine carcass tissue	1		6.82×10^2	24.3 (20.4)A
	2		3.50×10^0	12.5 (17.7)A
	3		4.13×10^1	0 (0)A
	4		1.23×10^2	9.5 (13.4)A
	5		1.24×10^2	27.8 (39.3)A
	6		7.23×10^3	46.3 (13.5)A
Bovine carcass sponge samples	1		2.18×10^4	76.7 (4.2)A
	2		1.66×10^3	42.8 (0.8)B
	3		2.60×10^1	28.8 (12.4)B
	4		3.20×10^2	75.0 (3.2)A
	5		9.13×10^2	76.6 (0)A
Bovine feces	1	1:10	3.24×10^5	44.1 (1.7)A
	2		6.20×10^6	54.3 (10.3)A
	3		4.38×10^6	39.4 (6.3)A
	4		4.21×10^5	11.6 (7.8)B,D
	5		2.99×10^6	32.1 (19.4)A,D
	1	1:100	6.87×10^4	97.9 (0.1)C
	2		6.61×10^5	98.5 (0.1)C
	3		3.67×10^5	99.4 (0.2)C
	4		3.91×10^4	93.6 (1.6)C
	5		2.35×10^5	42.7 (11.6)A
	1	1:1,000	8.34×10^3	99.5 (0.3)C
	2		5.08×10^4	99.5 (0.0)C
	3		7.07×10^4	99.6 (0.3)C
	4		3.64×10^3	99.4 (0.1)C
	5		2.96×10^4	94.1 (0.6)C

[a] Data presented are average of duplicate samples.
[b] Values followed by the same letter within the same sample type are not significantly different ($P \leq 0.05$).

In comparison to carcass surface tissue, however, adherence from sponge samples was higher and less variable. In three of the five sponge samples analyzed, adherence was 75.0% or greater. The lower adherence values seen with these two types of carcass samples may be due to the use of BPW-T as the suspending solution for these samples, although in preliminary testing, *E. coli* ATCC 25922 was recovered at percent adherence levels comparable to those observed with adsorption buffer, when inoculated into bovine carcass sponge samples taken using BPW-T as the sponge solution (data not shown).

Conclusions. For use in rapid microbial detection, a bacterial concentration method should be simple and fast, and it should recover microorganisms, particularly dilute microorganisms, quantitatively and in a viable state. Our investigation for use in bacterial concentration indicates that HA concentration can meet these criteria. Kinetic studies utilizing *E. coli* ATCC 25922 revealed that maximum adherence to HA takes place within 5 min, and comparable percent adherence values within this time frame were seen for both high (10^9) and low (10^3) concentrations of cells. In addition, fluorescent viability staining showed that bacterial cells adsorbed to HA remain viable. High percent adherence to HA was observed for several different species of both spoilage and pathogenic food-borne bacteria. The potential concentration of total bacteria that HA may afford would be advantageous in the microbial analysis of a food product, in that often there is more than one target species for which subsequent detection is needed. Additional studies planned include the demonstration of the utility of HA concentration of bacteria to increase the speed of microbial detection.

REFERENCES

1. **Abbott, A., and M. L. Hayes.** 1984. The conditioning role of saliva in streptococcal attachment to hydroxyapatite surfaces. J. Gen. Microbiol. **130:**809–816.
2. **Anonymous.** 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) systems; final rule. United States Department of Agriculture. Food Safety and Inspection Service. 9 CFR part 304, et al. Fed. Regist. **61:**38805–38989.
3. **Beem, J. E., C. G. Hurley, W. E. Nesbitt, D. F. Croft, R. G. Marks, J. O. Cisar, and W. B. Clark.** 1996. Fimbrial-mediated colonization of murine teeth by *Actinomyces naeslundii*. Oral Microbiol. Immunol. **11:**259–265.
4. **Bernardi, G., M. G. Giro, and C. Gaillard.** 1972. Chromatography of polypeptides and protein on hydroxyapatite columns. Some new developments. Biochim. Biophys. Acta **278:**409–420.
5. **Blackburn, C. D. W., L. M. Curtis, L. Humpheson, and S. B. Petitt.** 1994. Evaluation of the Vitek Immunodiagnostic Assay System (VIDAS) for the detection of *Salmonella* in foods. Lett. Appl. Microbiol. **19:**32–36.
6. **Brooks, T. L.** 1981. Hydroxylapatite. Calbiochem Brand Biochemicals, San Diego, Calif.
7. **Clark, W. B., L. L. Bammann, and R. J. Gibbons.** 1979. Comparative estimates of bacterial affinities and adsorption sites on hydroxyapatite surfaces. Infect. Immun. **19:**846–853.
8. **Clark, W. B., and R. J. Gibbons.** 1977. Influence of salivary components and extracellular polysaccharide synthesis from sucrose on the attachment of *Streptococcus mutans* 6715 to hydroxyapatite surfaces. Infect. Immun. **18:** 514–523.
9. **Clark, W. B., M. D. Lane, J. E. Beem, S. L. Bragg, and T. T. Wheeler.** 1985. Relative hydrophobicities of *Actinomyces viscosus* and *Actinomyces naeslundii* strains and their adsorption to saliva-treated hydroxyapatite. Infect. Immun. **47:**730–736.
10. **Cowan, M. M., K. G. Taylor, and R. J. Doyle.** 1987. Energetics of the initial phase of adhesion of *Streptococcus sanguis* to hydroxylapatite. J. Bacteriol. **169:**2995–3000.
11. **Gibbons, R. J., I. Etherden, and E. C. Moreno.** 1985. Contribution of stereochemical interactions in the adhesion of *Streptococcus sanguis* C5 to experimental pellicle. J. Dent. Res. **64:**96–101.
12. **Hillman, J. D., J. van Houte, and R. J. Gibbons.** 1970. Sorption of bacteria to human enamel powder. Arch. Oral Biol. **15:**899–903.
13. **Liljemark, W. F., and C. Bloomquist.** 1996. Human oral microbial ecology and dental caries and periodontal diseases. Crit. Rev. Oral Biol. Med. **7:** 180–198.
14. **Nesbitt, W. E., R. J. Doyle, K. G. Taylor, R. H. Staat, and R. R. Arnold.** 1982. Positive cooperativity in the binding of *Streptococcus sanguis* to hydroxylapatite. Infect. Immun. **35:**157–165.
15. **Payne, M. J., and R. G. Kroll.** 1991. Methods for the separation and concentration of bacteria from foods. Trends Food Sci. Technol. **12:**315–319.
16. **Rölla, G., and B. Melsen.** 1975. Desorption of protein and bacteria from hydroxyapatite by fluoride and monofluorophosphate. Caries Res. **9:**66–73.
17. **Siragusa, G. R., C. N. Cutter, W. J. Dorsa, and M. Koohmaraie.** 1995. Use of a rapid microbial ATP bioluminescence assay to detect contamination on beef and pork carcasses. J. Food Prot. **58:**770–775.
18. **Swaminathan, B., and P. Feng.** 1994. Rapid detection of food-borne pathogenic bacteria. Annu. Rev. Microbiol. **48:**401–426.
19. **Tian, H., T. Miyamoto, T. Okabe, Y. Kuramitsu, K.-I. Honjoh, and S. Hatano.** 1996. Rapid detection of *Salmonella* spp. in foods by combination of a new selective enrichment and a sandwich ELISA using two monoclonal antibodies against dulcitol 1-phosphate dehydrogenase. J. Food Prot. **59:** 1158–1163.
20. **Weerkamp, A. H., and B. C. McBride.** 1980. Adherence of *Streptococcus salivarius* HB and HB-7 to oral surfaces and saliva-coated hydroxyapatite. Infect. Immun. **30:**150–158.
21. **Wheeler, T. T., W. B. Clark, M. D. Lane, and T. E. Grow.** 1983. Influence of physicochemical parameters on adsorption of *Actinomyces viscosus* to hydroxyapatite surfaces. Infect. Immun. **39:**1095–1101.