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Blocking nonspecific adsorption of native food-borne microorganisms by immunomagnetic beads with ι-carrageenan

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Abstract—We present herein the partitioning characteristics of anti-*Salmonella* and anti-*Escherichia coli* O157 immunomagnetic beads (IMB) with respect to the nonspecific adsorption of several nontarget food-borne organisms with and without an assortment of well-known blocking agents, such as casein, which have been shown to be useful in other immunochemical applications. We found several common food-borne organisms that strongly interacted with both types of IMB, especially with anti-*Salmonella* form (av $\Delta G^0 = -20 \pm 4\,\mathrm{kJ\,mol^{-1}}$) even in the presence of casein [1% (w/v): $\Delta G^0 = -18 \pm 3\,\mathrm{kJ\,mol^{-1}}$; $\Delta\Delta G^0 \sim -2\,\mathrm{kJ\,mol^{-1}}$]. However, when one of the most problematic organisms (a native K12-like *E. coli* isolate; $\Delta G^0 = -19 \pm 2\,\mathrm{kJ\,mol^{-1}}$) was tested for nonspecific binding in the presence of ι-carrageenan (0.03–0.05%), there was an average decline of ca. 90% in the equilibrium capture efficiency ξ ($\Delta G^0 = -11 \pm 4\,\mathrm{kJ\,mol^{-1}}$; $\Delta\Delta G^0 \sim -8\,\mathrm{kJ\,mol^{-1}}$). Other anionic polysaccharides (0.1% κ-carrageenan and polygalacturonic acid) had no significant effect (av $\Delta G^0 = -19 \pm 1\,\mathrm{kJ\,mol^{-1}}$; $\Delta\Delta G^0 \sim 0\,\mathrm{kJ\,mol^{-1}}$). Varying ι-carrageenan from 0% to 0.02% resulted in ξ significantly diminishing from 0.69 (e.g., 69% of the cells captured; $\Delta G^0 = -19 \pm 3\,\mathrm{kJ\,mol^{-1}}$) to 0.05 ($\Delta G^0 = -11 \pm 2\,\mathrm{kJ\,mol^{-1}}$; $\Delta\Delta G^0 \sim -9\,\mathrm{kJ\,mol^{-1}}$) at about 0.03% ι-carrageenan where ξ leveled off. An optimum blocking ability was achieved with 0.04% ι-carrageenan suspended in 100 mM phosphate buffer. We also demonstrated that the utilization of ι-carrageenan as a blocking agent causes no great loss in the IMBs capture efficiency with respect to the capture of its target organisms, various salmonellae. Published by Elsevier Ltd.

Keywords: ι-Carrageenan; κ-Carrageenan; *Salmonella* Enteritidis; *Escherichia coli*; Gram-negative bacteria; Nonspecific adsorption; Binding; Immunomagnetic beads; IMB

1. Introduction

The isolation and concentration of diverse analytes with magnetic particles has been available for more than 25 years.[1,2] More recently, this partitioning technology was greatly enhanced with the ability to produce monodisperse, uniformly dense, spherical particles of small diameter (ca. 1–100 μm).[2] The manufacture of such uniform beads with imbedded magnetite resulted in the property of 'super-paramagnetism' causing them to be attracted toward a permanent magnet without preserving any magnetism upon removal of the applied field.[3] This latter characteristic allows magnetic particles to be easily partitioned from the diluent, washed, and resuspended for analysis.[4] Coating magnetic beads with proteins or other substances with assorted reactive functional groups, allows for the covalent attachment of capture polymers,[3] such as lectins or antibodies, onto the surface. Magnetic particles labeled with antibodies are referred to as immunomagnetic beads (IMBs).[5]

Conventionally, the initial steps in food-borne pathogen detection involves 'pre-enrichment',[6] which aids in the recovery of injured cells, and 'selective enrichment', which encourages growth of the pathogen while restricting the growth of other organisms. Using IMBs or other magnetic separation (MS) technologies, various workers[7–11] have established that this method of

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concentrating pathogens might replace selective enrichment. However, in many food products, disease-causing bacteria such as *Salmonella* and *E. coli* O157:H7 make up only a tiny fraction of an extensive, mainly benign, population of microbes. Since many bacteria adhere to manifold surfaces,[12–16] nonpathogenic organisms, which exist at high levels in certain food, may nonspecifically adhere to IMBs and thereby diminish their selective enrichment role in detection.

Because of the above, we were interested in determining if any blocking agents[17] exist that might ameliorate the nonspecific adhesion of bacteria onto IMBs and other capture surfaces. Most blocking agents have been developed for immunoassay applications[18–22] and are made from either nonspecific sera, detergents, milk proteins, bovine serum albumin (BSA), ovalbumin, gelatin, or some mixture derived therefrom.[17,21,23] These blocking reagents increase an immunoassay's signal-to-noise ratio by interacting with all solid-phase components and 'quenching'[20] the nonspecific binding of reporter antibodies onto these surfaces. Thus, we set about determining if traditional blocking buffers could be utilized to minimize the nonspecific binding of non-target (NT) organisms by anti-*Salmonella* and anti-*E. coli* 0157 IMBs. We have also investigated several neutral and anionic oligo- and polysaccharides for their effectiveness in reducing the nonspecific adsorption of our most problematic organism to anti-*Salmonella* IMBs.

2. Results and discussion

2.1. Modeling NT interactions with IMBs

Anti-*Salmonella* IMB capture efficiency (E) of a native *E. coli* O135NM-like (Experimental) organism was determined by varying either IMB concentration ([IMB]) or sample mixing time (τ_{mix}) and enumerating captured NT cells. The value of E is merely the ratio of captured cells to the total. As shown previously,[24] we observed (Fig. 1) that E varied with either τ_{mix} (fixed $[IMB] = 8.3 \times 10^6\,IMB\,mL^{-1}$; total organism concentration, or Δ_{total}, no greater than 10^3 cells mL^{-1}; Fig. 1A) or [IMB] (fixed $\tau_{mix} = 30$ min; Fig. 1B) as a pseudo first-order process (Eq. 1) with the rate constant equal to the product of an apparent IMB mass transport (γ, Eq. 2; $r_{IMB} \sim 2.4 \pm 0.3\,\mu m$) and concentration ([IMB]) terms[24]

$$E = \xi(1 - \exp[-\gamma[IMB]\tau_{mix}]), \quad (1)$$

$$\gamma = \frac{2g\pi r_{IMB}^4(\rho_{IMB} - \rho)}{9\eta}. \quad (2)$$

In Eq. 1, ξ is the calculated equilibrium value (or the extent of capture; value varying from 0 to 1) of the observed capture efficiency E at saturating [IMB] or long τ_{mix}. In Eq. 2, g is the gravitational acceleration constant (9.80665 m s^{-2}), r_{IMB} is the apparent hydrodynamic[24] radius (ca. 2.4 μm), ρ_{IMB} is the IMB density (ca. 1.3 g cm^{-3}) while ρ (ca. 1 g cm^{-3}) and η (ca. 0.89 g m^{-1} s^{-1}) are the buffer density and viscosity, respectively. These data demonstrate that NT binding to anti-*Salmonella* IMBs is identical to this same process[24] occurring with the IMB's intended target organism. However, unlike *Salmonella*, the IMB–NT binding process[24] saturates at ca. 10^7 beads mL^{-1}, whereupon only about 18–22% (average $\xi = 0.18 \pm 0.04$) of the population of the O135NM-like cells get captured (Fig. 1B). Since the NT capture process saturates at low levels of [IMB] and short τ_{mix}, these results argue that ξ for NT adsorption is related exclusively to the NT organism's cell surface (e.g., outer cell wall and associated flagellae/pilli) heterogeneity within the tested population. Thus, for these native *E. coli* O135NM-like cells, only about 20% ($\xi \sim 0.2$) of the population have some outer

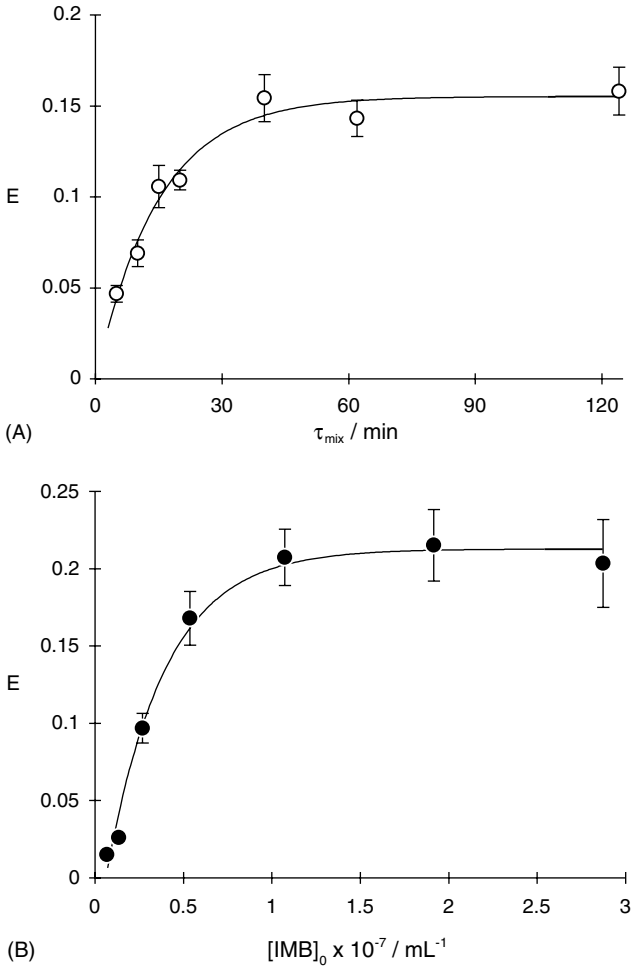

Figure 1. Dependency of capture efficiency of a native *E. coli* O135NM-like organism on anti-*Salmonella* IMB solution mixing time ($[IMB] = 8.3 \times 10^6$ beads mL^{-1}) and IMB concentration ($\tau_{mix} =$ 30 min). The curves were drawn with appropriate fits[42] to Eqs. 1 and 2.

membrane characteristic that allows them to tightly adhere upon collision with the anti-*Salmonella* IMB.

2.2. The nonspecific capture of other NT organisms by IMBs

Results for the anti-*Salmonella* and anti-*E. coli* O157 IMBs ability to nonspecifically bind (±casein) various NTs (Experimental) are shown in Tables 1 and 2. These data illustrate that, of the eight tested native NTs, four showed relatively large affinities (ξ ranging from 0.72 to 0.99; ΔG^0 − 20 to −33 kJ mol^{-1}) for the anti-*Salmonella* type IMBs. Neither of the gram-positive organisms (*Enterococcus* or *Listeria*) showed any noteworthy affinity for either IMB. However, two of the most problematic gram-negative bacteria (*Acinetobacter* and *Klebsiella*) did respond to the presence of the well-known blocking agent[23] casein by reductions in equilibrium capture efficiencies of 27% and 51%, respectively. Only *Acinetobacter* and a K12-like *E. coli* (Table 2) had elevated levels of nonspecific binding to both types of IMBs. Of the four NT organisms with a high level of IMB interaction, only the native K12-like *E. coli* did not respond significantly to casein. For this reason we chose this K12-like (ECK12) alone to use as the model organism for further assessment of potential blocking agents. Note that the tested IMBs displayed the expected high levels of capture of the targeted organisms *E. coli* O157:H7 and *Salmonella* Enteritidis (e.g., $\xi \sim 0.99$; $\Delta G^0 \sim -28$ kJ mol^{-1}; Table 2).

2.3. Screening for blocking activity using ECK12

Table 3 exhibits results for various traditional blocking agents and other substances with regard to the amelioration of ECK12 capture by anti-*Salmonella* IMBs. Interestingly, most of the common blocking buffers (casein, Super Block, Sea Block, Tween-20) inhibited the binding of ECK12 by no more than 3% (average $\Delta\xi = -6\%$ and $\Delta\Delta G^0 = -0.01$ kJ mol^{-1}). Because of the demonstrated poor blocking activity of conventional reagents, we chose to test several anionic and neutral oligo- and polysaccharides (Table 3). We chose to assess κ- and ι-carrageenan because the κ-form has been shown to reduce[15] the binding of collagen I to *E. coli* O157:H7. We chose to test various cyclodextrins because we felt they might bind to some relatively hydrophobic bacterial outer membrane components[25–31] thereby disrupting NT binding. Table 3 shows that the ι-carrageenan control (PBS) had a $\xi \sim 0.64$ ($\Delta G^0 \sim -19$ kJ mol^{-1}) for ECK12 by the anti-*Salmonella* IMBs, whereupon the addition of only 0.3 mg ι-carrageenan per milliter of diluent induced a 91% drop in the original binding capacity of the beads ($\Delta G^0 \sim -11$ kJ mol^{-1}; $\Delta\Delta G^0 \sim -8$ kJ mol^{-1}). This latter finding was a level of NT blocking not approached by even the best of the

Table 1. Ability of various nontarget organisms to bind to IMBs as measured by the equilibrium binding efficiency (ξ), change in ξ with casein ($\Delta\xi$), ΔG^0, and $\Delta\Delta G^0$ (change in ΔG^0 with casein)

Tested organism	Anti-*Salmonella* IMB						Anti-*E. coli* O157 IMB					
	ξ		$\Delta\xi$ (%)	ΔG^0		$\Delta\Delta G^0$	ξ		$\Delta\xi$ (%)	ΔG^0		$\Delta\Delta G^0$
	+Casein	−Casein		+Casein	−Casein		+Casein	−Casein		+Casein	−Casein	
Klebsiella[a] (6 reps)	0.350 ± 0.082	0.718 ± 0.122	51	−16.047	−19.625	−3.578	0.021 ± 0.031	0.187 ± 0.197	89	−8.624	−14.088	−5.464
Enterococcus (3 reps)	0.009 ± 0.001	0.004 ± 0.001	−125	−6.644	−4.765	1.879	0.011 ± 0.001	0.004 ± 0.004	−175	−7.111	−4.765	2.346
Listeria monocytogenes (3 reps)	0.004 ± 0.000	0.024 ± 0.002	83	−4.765	−8.938	−4.173	0.003 ± 0.001	0.019 ± 0.003	84	−4.100	−8.389	−4.288
Acinetobacter (3 reps)	0.724 ± 0.024	0.986 ± 0.004	27	−19.694	−27.272	−7.578	0.736 ± 0.018	0.984 ± 0.003	25	−19.834	−26.959	−7.125
Hafnia (3 reps)	0.237 ± 0.009	0.297 ± 0.027	20	−14.780	−15.488	−0.708	0.032 ± 0.004	0.054 ± 0.002	41	−9.620	−10.878	−1.258
Proteus (3 reps)	0.066 ± 0.007	0.190 ± 0.019	65	−11.369	−14.133	−2.763	0.035 ± 0.004	0.104 ± 0.011	66	−9.833	−12.512	−2.679
Aeromonas (3 reps)	0.001 ± 0.001	0.031 ± 0.004	97	−1.565	−9.544	−7.979	0.019 ± 0.002	0.511 ± 0.024	96	−8.401	−17.574	−9.173

[a] Table 4 for full epithet.

Table 2. Ability of various nontarget and target organisms to bind to IMBs as measured by the equilibrium binding efficiency (ξ), change in ξ with casein ($\Delta\xi$), ΔG^0, and $\Delta\Delta G^0$(change in ΔG^0 with casein)

Tested organism	Anti-*Salmonella* IMB						Anti-*E. coli* O157 IMB					
	ξ		$\Delta\xi$ (%)	ΔG^0		$\Delta\Delta G^0$	ξ		$\Delta\xi$ (%)	ΔG^0		$\Delta\Delta G^0$
	+Casein	−Casein		+Casein	−Casein		+Casein	−Casein		+Casein	−Casein	
E. coli K12, native (8 reps)	0.750 ± 0.123	0.733 ± 0.092	−2	−20.003	−19.799	0.204	0.249 ± 0.169	0.251 ± 0.159	1	−14.930	−14.954	−0.025
E. coli O135NM, native (3 reps)	0.388 ± 0.020	0.221 ± 0.015	−76	−16.423	−14.571	1.852	0.065 ± 0.005	0.054 ± 0.008	−20	−11.332	−10.878	0.454
E. coli K12, collection (3 reps)	0.254 ± 0.024	0.562 ± 0.022	55	−14.991	−18.047	−3.056	0.068 ± 0.012	0.246 ± 0.025	72	−11.443	−14.893	−3.450
E. coli O157:H7, collection (−)	0.001	0.001	0	−1.565	−1.986	−0.420	0.989	0.992	0	−27.834	−28.574	−0.740
Salmonella Enteritidis collection (−)	0.994	0.983	−1	−29.092	−26.845	2.247	0.018	0.017	−10	−8.300	−8.086	0.214

immunochemical blocking agents (bovine serum albumin $\Delta\xi \sim 9\%$, $\Delta G^0 \sim -18\,\mathrm{kJ\,mol^{-1}}$, and $\Delta\Delta G^0 \sim -1\,\mathrm{kJ\,mol^{-1}}$). The most effective blocking agent next to ι-carrageenan was the κ-form ($\Delta\xi \sim -13\%$; $\Delta G^0 \sim -18\,\mathrm{kJ\,mol^{-1}}$; $\Delta\Delta G^0 \sim -1\,\mathrm{kJ\,mol^{-1}}$). None of the cyclodextrans or other anionic polysaccharides performed particularly well (average $\Delta\xi \sim -6\%$ and $\Delta\Delta G^0 \sim 0.43\,\mathrm{kJ\,mol^{-1}}$).

2.4. ι-Carrageenan dose–response

If the anionic polysaccharide ι-carrageenan operates by blocking binding sites on ECK12 surfaces, then one should observe transitional levels of effective binding, as measured by ξ, associated with intermediate concentrations of the polysaccharide, and the blocking effect should eventually saturate. Such an experiment is presented in Figure 2, whereupon the equilibrium capture efficiency (ξ) of anti-*Salmonella* IMBs for ECK12 is plotted as a function of ι-carrageenan dose [0–0.05% (w/v)]. In PBS alone, about 70% of ECK12 was projected to be captured ($\xi = 0.7$; 3 h of mixing at 4 °C; see Experimental). At about 0.015% ι-carrageenan ξ was seen to diminish ca. 23% to a ξ value of ca. 0.53. At 0.02% ξ dropped to 0.14 and thereafter saturated to a ξ level of 0.04 ($\Delta\xi$ ca. 94%) between 0.03% and 0.05% ι-carrageenan. Thus, as was seen previously (Table 3), ι-carrageenan concentrations of between 0.03% and 0.05% induced a perturbation in the equilibrium capture efficiency $\Delta\xi$ of greater than 90%.

2.5. The role of Na^+ in ι-carrageenan blocking

Because ionic strength,[32] particularly when modulated with Na^+,[12] has been shown to have a strong positive effect on the adsorption of some bacteria to diverse surfaces, and because our ι-carrageenan is highly electronegative at pH 6.8, we sought to investigate the effect of NaCl and phosphate anion activity (e.g., $[H_2PO_4^-]$, $[NaHPO_4^-]$, $[HPO_4^{2-}]$) on the ability of ι-carrageenan to block ECK12 binding to anti-*Salmonella* IMBs. Figure 3A shows a plot of ξ for ECK12 as a function of NaCl in either 100 or 20 mM PB. All controls (average of 20 and 100 mM PB + variable NaCl: 0% ι-carrageenan) remained relatively constant ($\xi \sim 0.6$). The 100-mM PB (+0.04% ι-carrageenan) treatments also were relatively constant ($\xi \sim 0.1 \pm 0.01$; $\Delta\xi \sim 81\%$) regardless of the NaCl level. However, the 20-mM PB set of data showed a linear decrease in blocking ability (ξ increasing from ca. 0.2–0.5 between 0% and 0.8% NaCl). Upon plotting ξ with total ionic strength (Fig. 3B), it becomes clear that Na^+ has a ξ-reversing effect mainly at a low phosphate concentration. Since ι-carrageenan appears to be insoluble in either buffer system, these results suggest that the polysaccharides microgel structure might be affected under these conditions.

Table 3. Ability of a native *E. coli* K12-like organism to bind to anti-*Salmonella* IMBs as measured by the equilibrium binding efficiency (ξ), change in ξ with tested diluent ($\Delta\xi$), ΔG^0, and $\Delta\Delta G^0$(change in ΔG^0 with diluent)

Tested component	ξ		$\Delta\xi$ (%)	ΔG^0		$\Delta\Delta G^0$
	+	−		+	−	
0.03% ι-Carrageenan (12 reps)	0.058 ± 0.023	0.639 ± 0.064	91	−11.047	−18.786	−7.739
0.1% κ-Carrageenan (6 reps)	0.578 ± 0.328	0.664 ± 0.162	13	−18.195	−19.038	−0.843
1% BSA (2 reps)	0.600 ± 0.214	0.661 ± 0.087	9	−18.407	−19.009	−0.602
2 mM Hydroxyethyl-β-CD (2 reps)	0.560 ± 0.008	0.610 ± 0.100	8	−18.026	−18.502	−0.476
0.1% Gellan (2 reps)	0.631 ± 0.001	0.677 ± 0.067	7	−18.713	−19.177	−0.465
1% Casein (6 reps)	0.759 ± 0.122	0.784 ± 0.053	3	−20.109	−20.441	−0.332
2 mM α-CD (2 reps)	0.587 ± 0.028	0.592 ± 0.099	1	−18.278	−18.333	−0.055
1% 'Super block' (2 reps)	0.651 ± 0.032	0.658 ± 0.004	1	−18.907	−18.979	−0.072
1% 'Sea block' (2 reps)	0.656 ± 0.127	0.658 ± 0.004	0	−18.963	−18.979	−0.016
0.1% PGA (4 reps)	0.728 ± 0.143	0.677 ± 0.067	−7	−19.737	−19.177	0.560
0.25% HP-β-CD (−)	0.749	0.684	−10	−19.991	−19.251	0.739
0.1% Sulfated-β-CD (2 reps)	0.809 ± 0.080	0.611 ± 0.027	−32	−20.802	−18.513	2.289
0.1% (2 reps each)						
Tween-20	0.498 ± 0.143	0.388 ± 0.033	−28	−17.451	−16.426	1.026
Tween-40	0.7844 ± 0.0923	—	−102	−20.447	—	4.021
Tween-80	0.860 ± 0.0175	—	−121	−21.652	—	5.226

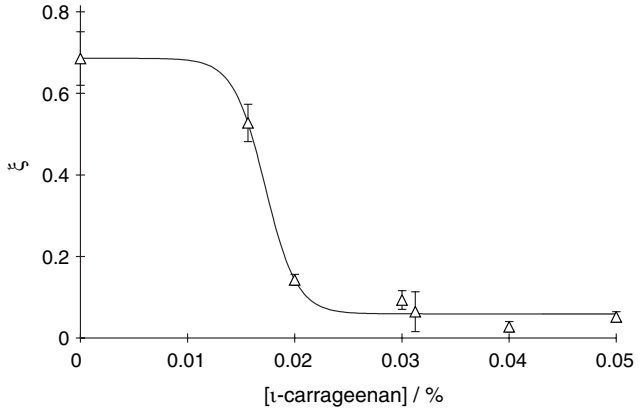

Figure 2. Effect of ι-carrageenan concentration (% w/v) on the capture equilibrium of a native *E. coli* K12-like (ECK12) organism binding to anti-*Salmonella* IMBs at 4 °C in phosphate-buffered saline.

2.6. ι-Carrageenan effect on *Salmonella* capture

Figure 4 shows a bar plot of ξ representing ECK12 and *Salmonella* Enteritidis capture as a function of blocking buffers containing either PBS (control, 100 mM) or PBS + 0.03% ι-carrageenan. The ECK12 experiments showed an average ξ of ca. 0.11 ± 0.02 (control 0.69 ± 0.17) for ι-carrageenan, whereas for *Salmonella* ξ averaged 0.92 ± 0.05 (control 0.96 ± 0.02). Thus ι-carrageenan, at least at the levels we have utilized, appears to have only a minor effect on the capture of the target organism.

3. Conclusions

We have observed and modeled the equilibrium affinities of several nontarget food-borne organisms for anti-*Salmonella* and anti-*E. coli* O157 IMBs with and without an assortment of well-known blocking agents. In the course of this work, we have found several common food-borne organism that strongly interact with both IMB types, even in the presence of casein. However, when our native ECK12 isolate was tested for IMB capture in the presence of ι-carrageenan, there was an average decline of ca. 80–90% in the equilibrium capture efficiency ξ. Other anionic polysaccharides, such as κ-carrageenan or polygalacturonic acid, had small, or nil, blocking effects. The ι-carrageenan blocking effect was apparently related to the polysaccharides anionic structure as changing the buffers ionic strength tended to result in larger ξ values when NaCl was present. We have also demonstrated that the utilization of ι-carrageenan as a blocking agent causes no significant loss in the anti-*Salmonella* IMBs capture efficiency with respect to the capture of its target organism.

4. Experimental

4.1. General

Brain–heart infusion broth (BHI) was obtained from Difco (Detroit, MI). Typically only serial dilutions estimated to contain 1000–2000 CFU mL^{-1} were utilized for nontarget (NT) binding studies and enumerated after further dilution (e.g., either 1:10- or 1:20-fold). Phosphate-buffered saline [PBS; 100 mM, 0.85% (w/v) NaCl, pH 6.8] was used as the base diluent throughout. All error terms presented in figures are statistics (ε)[24] based on the propagation of errors method.[33] All error terms presented in tables are standard deviations.

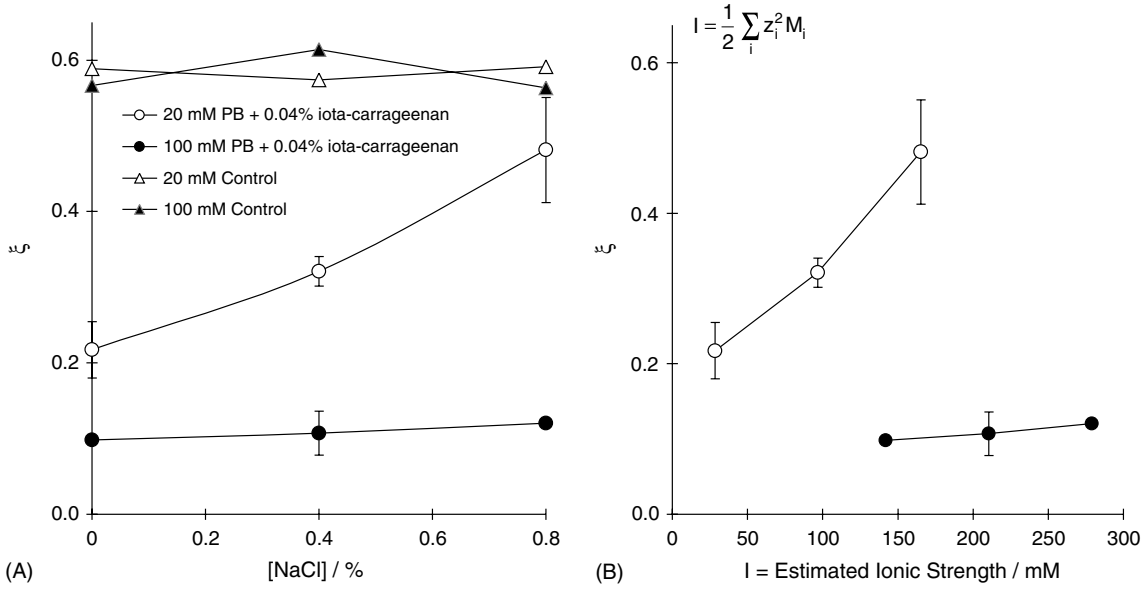

Figure 3. Effects of NaCl (% w/v) and PB ionic strength with and without 0.04% ι-carrageenan on anti-*Salmonella* IMBs ability to partition a native *E. coli* K12-like organism. (A) ξ Plotted as a function of NaCl concentration (% w/v). (B) ξ Plotted as a function of total ionic strength (equation in figure: z_i = charge of ionic species i; M_i molar concentration of species i).

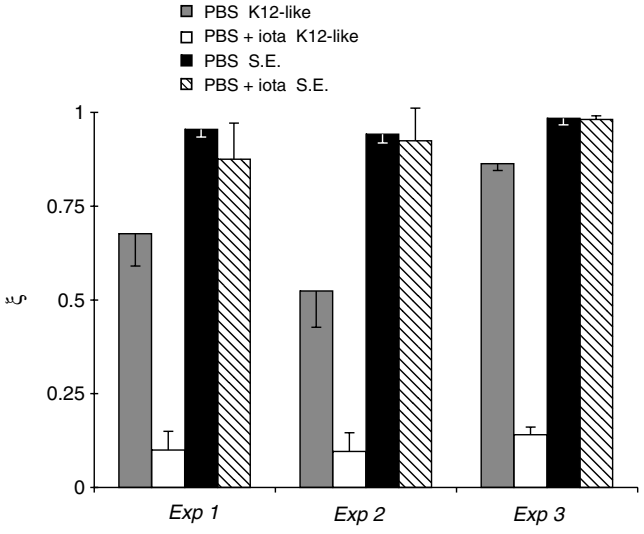

Figure 4. Dependency of anti-*Salmonella* IMB equilibrium binding affinity for both *Salmonella* Enteritidis and our native *E. coli* K12-like organism as a function of ι-carrageenan concentration (0.035% w/v) in phosphate-buffered saline.

4.2. Nontarget bacteria identification

All 'native' nontarget (NT) organisms on which we report were isolated from six Cornish game hens and one small 'organically raised' chicken (estimated ca. 2×10^7 total aerobic cells per chicken). Whole carcasses were washed with filter-sterilized PBS in a sterile bag, the wash was collected and filtered to remove debris, and pipetted into a sterile bottle. This carcass wash was used to inoculate BHI and incubated at 37 °C for ca. 16 h. The resulting BHI culture was serially diluted (1:10, from an estimated ca. 10^9 to 10^2 cells mL^{-1}; 1:2, from ca. 10^2 to 10^0 cells mL^{-1}), and 50 μL of each dilution pipetted into each well (150 μL BHI) of 96-well microtiter plates. Wells from the dilutions that displayed least amount of positive growth ($p \leqslant 30$) were sampled and streaked, and representative colonies were re-plated in order to secure a clone. Isolates were then gram stained, compared morphologically, and grouped based on these similarities.

Representative isolates were identified (Table 4) using the Vitek biochemical testing method (an array of individual growth tests ± some biochemical component) and a ribosomal DNA-MicroSeq sequence analysis (ca. 500-base PCR-amplified region of rDNA used for comparing to a large database on many different organisms) method. Only isolates shown to agree between the two methods of identification were used for further study. Of the original isolates, eight were utilized in IMB-binding studies after an initial screening for binding activity. We also tested *Salmonella* Enteritidis, *Escherichia coli* O157:H7, *E. coli* K12 (true serotype, nonpathogenic) and *Listeria monocytogenes* H7762 (pathogenic strain, obtained from Dr. L. Wonderling) from our culture collection. All the forenamed culture collection isolate identities were confirmed using the rDNA sequencing–bioinformatics method.

4.3. IMB·NT complex calculations

For the capture efficiency experiments depicted in Figure 1, six dilutions of our native *E. coli* 0135NM-like organism (400–700 CFU mL^{-1} diluted 1-, 0.5-, 0.25-, 0.125-, 0.0625-, and 0.03125-fold) each containing 7 mL

Table 4. Native chicken isolate identifications based upon rDNA sequence and biochemical tests

% Agreement	Ribosomal DNA sequence-based identification (Microseq PCR database)	% Agreement	Vitek (biochemical test array)
99.43	*Klebsiella pneumoniae rhinoscleromati*	99	*Klebsiella pneumoniae*
98.81	*Enterococcus faecalis* (gram+)	99	*Enterococcus faecalis*
98.77	*Hafnia alvei*	91	*Hafnia alvei*
99.72	*Proteus mirabilis*	96	*Proteus mirabilis*
99.81	*Aeromonas veroni*	90	*Aeromonas veronii biovar sobria*
98.47	*Acinetobacter baumannii*	99	*Acinetobacter calcoaceticus-baumannii* complex
>99	*E. coli* O135NM	98	*E. coli*
99.24	*E. coli* K12	98	*E. coli*

were dispensed into six sterile (Corning 9826-13: 13×100 mm) Pyrex screw-cap culture tubes. Because of this arrangement, each datapoint shown in Figure 1 was calculated from six single-dilution observations per treatment combination (i.e., 6 [NT] as a function of variable [IMB] or τ_{mix}). To each mL of test solution 1–43 μL (ca. 6.7×10^5 IMB μL^{-1}) of Dynal anti-*Salmonella* IMBs were added and mixed (τ_{mix} varied from 5–120 min on a Dynal 947.01 sample mixer set to 30 rpm) at ca. 4 °C. After mixing for τ_{mix} min, IMBs were magnetically isolated for 5 min (solution saved for analysis), washed with PBS and magnetically isolated again, and the wash was discarded. Sterile PBS (7 mL) was added back to each tube and vortexed to re-suspend the IMB-captured NT cells. Samples were kept on ice between use.

For all other nontarget (NT) binding experiments (1000–2000 cells mL^{-1}; for assays these were diluted further by ca. 1:10–1:20 for an estimated cell density of ca. 100 mL^{-1}), 2 (control + one for IMB treatment)×1.5 mL of serially diluted organism (PBS ± test blocking agent, pH 6.8) were dispensed into two sterile 2-mL Eppendorf micro-centrifuge tubes. For each IMB treatment, 18 μL (~1.2×10^7 beads) of Dynal anti-*Salmonella* or anti-*E. coli* O157 IMBs were added, and the samples mixed for 120–180 min (Dynal 947.01 sample mixer; 30 cycles min^{-1}) at ca. 4 °C. The IMBs were magnetically isolated 5 min (as recommended by the manufacturer; Dynal MPC-S high-field-strength magnet), with a gentle rocking motion, whereupon the supernatant was removed (Δ_{free}) and the IMBs washed with the appropriate PBS-based test blocking buffer and magnetically isolated, and the wash was discarded. The original volume (1.5 mL) of test blocking buffer was added back to each tube and vortexed to re-suspend the IMB-captured cells (Δ_{bound}). Samples were refrigerated between use. Any experiment where the control cell concentrations (Δ) differed significantly (e.g., $\pm t_{0.05}\varepsilon$) from $\Delta_{total}(\Delta_{bound}+\Delta_{free})$ were repeated. Capture efficiency (E) was calculated as

$$E=\frac{\Delta_{bound}}{\Delta_{total}}=\frac{\Delta_{bound}}{\Delta_{free}+\Delta_{bound}}. \quad (3)$$

4.4. Bacteria enumeration: 96-well most probable number (MPN) assays

Any MPN method[34–40] consists of taking a sample from a liquid source, making serial dilutions from this, introducing an aliquot of each of the dilutions into an appropriate culture medium and incubating samples at a suitable temperature in order to observe if any growth occurs (±turbidity). One of the most important steps in the application of the dilution method is calculating a statistically meaningful estimation of the cell concentration, or MPN, in the original sample. Most MPN calculations are based on the application of binomial probability theory, which depends upon two primary assumptions:[40] microbes are distributed randomly throughout the test fluid, and growth occurs if the aliquot used contains one or more organisms.

In order to obtain the greatest precision and accuracy, six dilution (i.e., the dilution factors $\Phi=1$, 0.5, 0.25, 0.125, 0.0625, 0.03125; the $\Phi=1$ dilution resulted from a 1:10- or 1:20-fold dilution of the original concentration), 96-well microtiter plate turbidity assays[34,41] were used for bacterial enumeration. For each Δ, Δ_{bound}, or Δ_{free} to be enumerated, 150 μL of sterile BHI was pipetted into every well of UV-irradiated 96-well microtiter plates (one plate per dilution) and 50 μL of inoculum introduced (test diluent–diluted NT cells; 0–100 mL^{-1}). All plating was performed in a Type II Vertical Microbiological Hood. Inoculated plates were stored in a sterile container and incubated overnight at 37 °C. After 16–18 h, the number of positive responses (p), based on turbidity, was recorded. Using this technique we observe a limit of detection of about 0.4 cells mL^{-1}.[34,35]

The MPN calculation we have utilized[35] takes advantage of the fact that by dividing $\partial_\Delta P_i$ by P_i (P_i is the binomial probability function for each ith dilution), summing this across all i dilutions, and iteratively varying Δ (Eq. 4, below) to obtain a near-zero sum, we achieve a final solution for Δ, which gives a maximum in $\Pi_i P_i$ which is the MPN

$$\Delta_{j+1} = \left| \Delta_j + x_j \sum_i^{\mathrm{I}} \frac{\partial_{\Delta} P_i}{P_i} \right| = \left| \Delta_j + x_j \sum_i^{\mathrm{I}} \left(p_i - n + \frac{p_i}{\exp[v\Phi_i \Delta] - 1} \right) v\Phi_i \right|. \quad (4)$$

By adding the total relative deviation of $\{\partial_{\Delta} P_i \div P_i\}$ from 0 to an initial estimate of $\Delta(\Delta_{j=0}; j$ iterations), we induce the calculation of a new value (Δ_{j+1}), which must be closer to the MPN. The x term is a scaling factor, which normalizes the summation to an appropriate level.[35]

4.5. Equilibrium calculations of nontarget bacterial (NT) adsorption to anti-*Salmonella* or anti-O157 immunomagnetic beads (IMB)

All our binding reactions (4 °C) were characterized by estimating the standard free-energy change of the system

$$\Delta G^0 = -RT \ln[K] \quad (5)$$

whereupon

$$K = \frac{\Delta_{\mathrm{bound}} C}{\Delta_{\mathrm{free}}[\mathrm{IMB}]} = \frac{\xi C}{(1-\xi)([\mathrm{IMB}] - \Delta_{\mathrm{total}}\xi)} = \frac{\xi C}{\Delta_{\mathrm{total}}\xi^2 - (\Delta_{\mathrm{total}} + [\mathrm{IMB}])\xi + [\mathrm{IMB}]}. \quad (6)$$

In this expression, ξ is the calculated equilibrium value (or the extent of capture; value from 0 to 1) of the observed capture efficiency $E(\tau_{\mathrm{mix}} = 2\text{–}3\,\mathrm{h}$; $[\mathrm{IMB}] = 8\times10^6\,\mathrm{mL}^{-1}$; $\Delta_{\mathrm{total}} \sim 2\times10^3\,\mathrm{cells\,mL}^{-1})$ whereupon

$$\xi = \frac{E}{1 - \exp[-\gamma[\mathrm{IMB}]\tau_{\mathrm{mix}}]}, \quad (7)$$

usually, E and ξ differ only by about 3–5% with long τ_{mix} (Fig. 1). Referring back to Eq. 6, we have corrected the observed bacteria ($\Delta_{\mathrm{total}} = \Delta_{\mathrm{free}} + \Delta_{\mathrm{bound}}$) levels by the factor C (1.58×10^{10}) by iteratively solving for the value of C which induced Eq. 5 to go near to zero (Fig. 5) as ξ approached the limit of detection. The parameter C is a term that converts the microbiological units of CFU per milliliter to units of activity. Thus, this empirically derived quantity implies that the concentration of bacteria in the standard state is ca. $10^{10}\,\mathrm{mL}^{-1}$. A pure colony of rod-shaped gram-negative bacteria similar in size to *Salmonella* Enteritidis would contain about $10^{12}\,\mathrm{mL}^{-1}$. In Figure 5 we have plotted all our C-modulated ΔG^0 values (Eqs. 5–7) as a function of ξ. These data graphically demonstrate that the corrected values of ΔG^0 has an asymptote near 0 as ξ approaches 0.

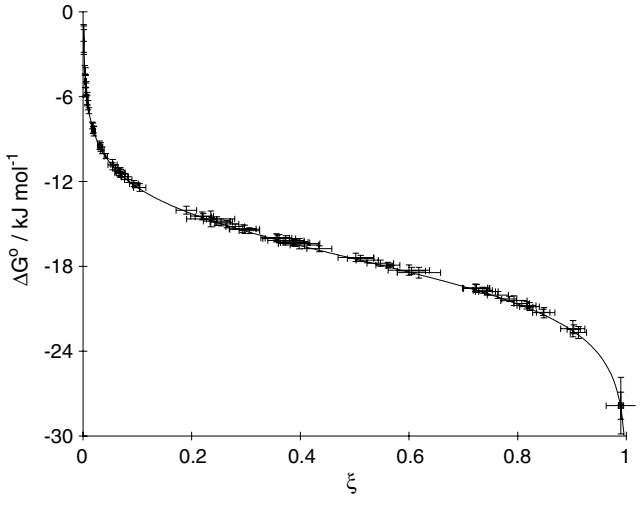

Figure 5. Plot of calculated ΔG^0 as a function of ξ. This figure demonstrates that the correction factor C in Eq. 6 induces ΔG^0 to approach 0 as ξ becomes small.

4.6. Preparation of test blocking buffer

All substances tested were either suspended or dissolved in PBS, pH 6.8. Casein, BSA, 'Super Block', 'Sea Block', Tween-20, -40, -80, and various cyclodextrins (CD: hydroxyethyl-β-CD, hydroxypropyl-β-CD, α-CD, sulfated-β-CD) were subjected to sterile filtration. ι-Carrageenan, κ-carrageenan, gellan, and polygalacturonic acid were autoclaved.

Neither ι-carrageenan nor κ-carrageenan (Sigma chemical company) go into solution at high ionic strengths. Therefore the carrageenan suspensions were sonicated 15–20 min while still warm to break up large particles. The amount of each test blocking agent are provided in the appropriate tables and figures.

5. Abbreviations

BHI	brain–heart infusion broth used in the MPN determination and growth of our target and nontarget organisms
ECK12	native (Table 4) K12-like *E. coli* used for most of the capture studies with anti-*Salmonella* IMBs
Δ	MPN-determined solution density of the tested target or NT bacterial cells: no subscript indicates the control, subscript 'bound' stands for the IMB-bound cell MPN, and subscript 'free' symbolizes the MPN of the supernatant after IMB capture and MS
E	capture efficiency defined as the ratio of bound-to-total bacteria
ξ	calculated extent of capture at equilibrium
IMB	immunomagnetic beads
MS	magnetic separation
[IMB]	initial IMB concentration before binding of target and NT organisms
τ_{mix}	mixing time; $\tau_{\mathrm{mix}} \sim 2\text{–}3$ h
n	total number of observations used per dilution in our MPN protocol
NT	nontarget organisms (see Table 4)

p number of positive (turbid) samples of growth out of n observations

PBS phosphate buffered saline: 100 mM sodium phosphate buffer (pH 6.8) + 0.85% NaCl

Φ_i dilution factor for the ith dilution; product of the various individual dilution factors $\phi_i =$ volume of the $(i-1)$th dilution used to make dilution $i \div$ total volume of diluent and v_{i-1}

v volume of sample (usually 50 μL) used to inoculate 150 μL of BHI in our MPN assay

i, j subscripts used as indices: i-dilutions and j-iterations used for denoting steps in iterative solving for the MPN

$\partial_x f(x)$ shorthand for $\frac{d}{dx} f(x)$; usually taken as a partial first derivative

K equilibrium partition coefficient (equilibrium constant) for the binding of bacteria with immunomagnetic beads

C correction factor to induce the calculated ΔG^0 to asymptote toward zero as ξ goes to zero; for the [IMB] used herein $C = 10^{10.2}$ (Fig. 5)

ΔG^0 observed standard (4 °C) free energy change of the IMB–NT interacting system (Fig. 5, Eqs. 5–7).

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