Eliminating the Carriage of *Salmonella enterica* Serovar Enteritidis in Domestic Fowls by Feeding Activated Charcoal from Bark Containing Wood Vinegar Liquid (Nekka-Rich)

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ABSTRACT The protective efficacy of activated charcoal containing wood vinegar liquid (Nekka-Rich) against intestinal infection with *Salmonella enterica* serovar Enteritidis was sought. In the present study, the adsorption effects of activated charcoal of Nekka-Rich on *S.* Enteritidis and normal bacterial flora in the intestine, *Enterococcus faecium*, were examined. *S.* Enteritidis was effectively adsorbed by activated charcoal of Nekka-Rich. On the other hand, activated charcoal of Nekka-Rich showed lower binding capacity to *E. faecium*. The effects of wood vinegar liquid included in Nekka-Rich on the growth of *S.* Enteritidis and normal bacterial flora in the intestine, *E. faecium* and *Bifidobacterium thermophilum*, were also assessed. Wood vinegar liquid had an inhibitory effect on the *S.* Enteritidis growth, whereas growth of *E. faecium* and *B. thermophilum* was enhanced by wood vinegar liquid. Furthermore, the protective effects of Nekka-Rich were evaluated in a challenge chicken model with *S.* Enteritidis. Chickens were fed a basal diet containing Nekka-Rich or immunized with commercially obtained *S.* Enteritidis vaccine and challenged with *S.* Enteritidis. Significantly less fecal excretion of *S.* Enteritidis was observed in chickens fed Nekka-Rich for 10 d after challenge. On d 15 after challenge, *S.* Enteritidis was not isolated from fecal samples. On the other hand, immunization of chickens with *S.* Enteritidis vaccine did not fully inhibit bacterial growth. Fecal excretion of *S.* Enteritidis was consistently observed in the vaccinated chickens after challenge. These results suggest that Nekka-Rich would be a good product for eliminating the carriage of *S.* Enteritidis in domestic fowl.

(*Key words*: activated charcoal, foodborne disease, *Salmonella*, probiotic, wood vinegar liquid)

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INTRODUCTION

Salmonella enterica serovar Enteritidis is now a common pathogen of many species of mammals and birds. It is the most common cause of bacterial gastroenteritis in humans; infection is usually associated with the ingestion of contaminated chicken eggs, egg products, or chicken meat (Rodrique et al., 1990; Telzak et al., 1990). Thus, methods including vaccination for reduction or elimination of the carriage of *S.* Enteritidis in poultry are needed to reduce the level of exposure to the pathogen in food and the environment.

Activated charcoal is a universal adsorbent because it can bind with variety molecules (Chandy and Sharma, 1998). It has been reported that activated charcoal is useful for removal of bacteria and bacterial toxins, both in vitro and in vivo (Drucker et al., 1977; Pegues et al., 1979; Du et al., 1987; Gardiner et al., 1993; Marks et al., 1998). Recently, we have also shown the usefulness of activated charcoal for removal of verotoxin-producing *Escherichia coli* and verotoxin (Naka et al., 2001). Moreover, it has also been reported that organic acids have an inhibitory effect on the growth of enteropathogenic bacteria (Anderson, 1992; Hsiao and Siebert, 1999; Nakai and Siebert, 2003). Nekka-Rich is activated charcoal made from the bark of an evergreen oak and contains wood vinegar liquid including organic acids. In Japan, it is approved as a feed additive by the Japanese Government. Therefore, we examined whether activated charcoal of Nekka-Rich could effectively adsorb *S.* Enteritidis, and whether wood vinegar liquid contained in Nekka-Rich is effective in the growth inhibition of *S.* Enteritidis. In addition, we also evaluated the ability of Nekka-Rich to protect against *S.* Enteritidis infection in chickens after challenge with the bacteria.

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Abbreviation Key: GAM = Gifu anaerobic medium.

MATERIALS AND METHODS

Chickens

Salmonella-free Hy-Line White Leghorn chickens (W36 strain) (8 wk old) were purchased from a commercial farm.[2] All chickens were maintained in our laboratory in a *Salmonella*-free environment and were fed with antibiotic-free feed. They were maintained according to the Standards Relating to the Care and Management of Experimental Animals of Japan.

Materials and Bacteria

Activated charcoal from the bark of an evergreen oak, wood vinegar liquid from the bark of an evergreen oak, and Nekka-Rich, a product that includes activated charcoal and wood vinegar liquid from the bark of an evergreen oak were purchased.[3] The *S.* Enteritidis strain 1227 was kindly provided by Y. Adachi.[4] The organism was originally isolated from chicks with salmonellosis. It was grown at 37°C for 72 h in static liquid colonization factor antigen medium (Evans et al., 1977) containing 5 m*M* KH_2PO_4 and 12 m*M* Na_2HPO_4. *Enterococcus faecium* (CL-5) and *Bifidobacterium thermophilum* (BL-4) were gifts from Y. Kodama[5] and were grown under anaerobic conditions in Gifu anaerobic medium (GAM) broth[6] at 37°C overnight.

Adsorption Test

This study was designed to test the usefulness of activated charcoal from the bark of an evergreen oak for *S.* Enteritidis adsorption. Approximately 4.8 to 6.3×10^6 cfu of *S.* Enteritidis in 1 mL of heart infusion broth[6] and activated charcoal from the bark of an evergreen oak (1, 3, 5, and 10 mg) were mixed together and incubated at 37°C for 1 h with gentle agitation. These mixtures were then centrifuged at $900 \times g$ for 5 min to remove activated charcoal from the bark. After centrifugation, an aliquot of supernatant was diluted and plated onto heart infusion agar[6] to determine the number of bacteria.

Activated charcoal from the bark of an evergreen oak was also tested for its capacity to bind normal bacterial flora in the intestines. We used *E. faecium* as model bacteria. An adsorption test was carried out using approximately 2.1 to 4.9×10^6 cfu of bacteria/mL in GAM broth as described above. After centrifugation for 5 min at $900 \times g$, an aliquot of upper phase was diluted and streaked onto BL agar[6] to calculate the number of bacteria.

Effect of Wood Vinegar Liquid on the Bacteria

The effects of wood vinegar liquid on bacterial growth were evaluated. *S.* Enteritidis (approximately 1.0 to 1.6×10^7 cfu of bacteria) was added to 10 mL of heart infusion broth containing 0, 0.25, 0.5, 1.0, or 2.0% wood vinegar liquid or acetic acid (pH 7.0) and then incubated at 37°C for 14 h with gentle agitation. After incubation, an aliquot of bacteria mixture was diluted and plated onto heart infusion agar to determine the number of bacteria.

Wood vinegar liquid was also tested for its effect on the growth of normal bacterial flora in the intestines. We used *E. faecium* and *B. thermophilum* as model bacteria. The test was carried out using approximately 3.7 to 10.0×10^6 cfu of bacteria in 10mL of GAM broth as described above. After incubation at 37°C for 14 h, an aliquot of bacteria mixture was diluted and streaked onto BL agar to calculate the number of bacteria.

Administration of Nekka-Rich and Challenge Inoculations

To study protection against *S.* Enteritidis in chickens by feeding Nekka-Rich, chickens were divided in 3 groups (8 chickens/group). For the challenge experiment with *S.* Enteritidis, chickens (12 wk old) were housed in individual cages in 2 vinyl isolators set in the infection room (4 chickens/isolator). Physical contact between chickens was prevented. Group A (untreated control) was inoculated orally with *S.* Enteritidis suspension. Group B was immunized intramuscularly with 0.5 mL of *S.* Enteritidis vaccine,[7] and immunization was repeated after 4 wk. One week after the booster immunization, chickens were orally challenged with bacterial suspension. Group C was provided feed containing 1% Nekka-Rich (wt/wt). One week later, chickens were orally challenged with *S.* Enteritidis suspension and then were given Nekka-Rich-supplemented feed for 16 d. Birds were challenged at 13 wk of age with 1×10^7 cfu bacteria.

Fresh fecal samples (approximately 2 to 3 g) were collected from each chicken on d 2, 5, 10, and 15 d after challenge. Sixteen days after challenge chickens were killed, and the visceral organs and intestinal contents of the duodenum, small intestine, cecum, and rectum regions were collected aseptically. All samples were weighed and suspended in sterile PBS at 2× dilution. They were then serially diluted 10-fold in PBS, and 100 μL of each dilution was spread onto mannitol lysine crystal violet brilliant green agar plates.[6] After cultivation at 37°C for 24 h, dark colonies of *S.* Enteritidis on the plates were counted. Bacteria were identified by the slide agglutination test using O9 group-specific antiserum.[8]

PCR

Salmonella Enteritidis DNA in the intestinal contents and visceral organs was amplified by PCR as described by Lampel et al. (1996). One-milliliter aliquots of organ

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[5]Immunology Research Institute in Gifu, Gifu, Japan.
[6]Nissui Pharmaceutical Co., Ltd., Tokyo.
[7]GHEN Co., Gifu, Japan.
[8]Denka Seiken Co., Tokyo.

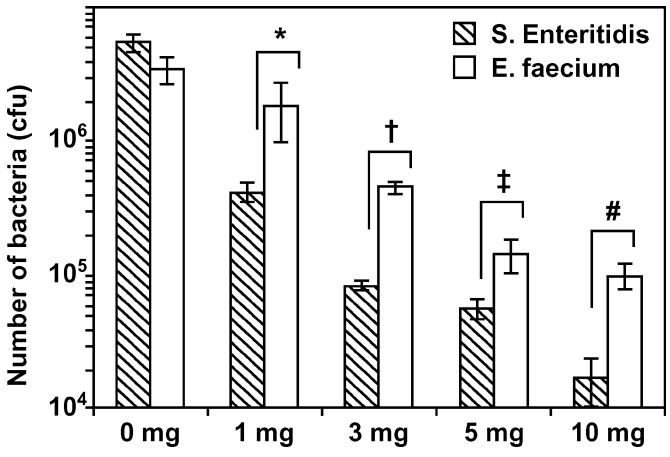

FIGURE 1. The capacity of activated charcoal to adsorb the bacteria. An adsorption experiment was performed with *Salmonella enterica* serovar Enteritidis ($5.53 \pm 0.74 \times 10^6$ cells) and *Enterococcus faecium* ($3.51 \pm 1.38 \times 10^6$ cells) using various amounts of activated charcoal (1, 3, 5, and 10 mg). The number of unadsorbed bacteria was determined as described in the materials and methods section. Data are expressed as means ± SE of triplicate experiments. *$P < 0.031$, †$P < 0.0001$, ‡$P < 0.023$, and #$P < 0.003$ between *S.* Enteritidis and *E. faecium*.

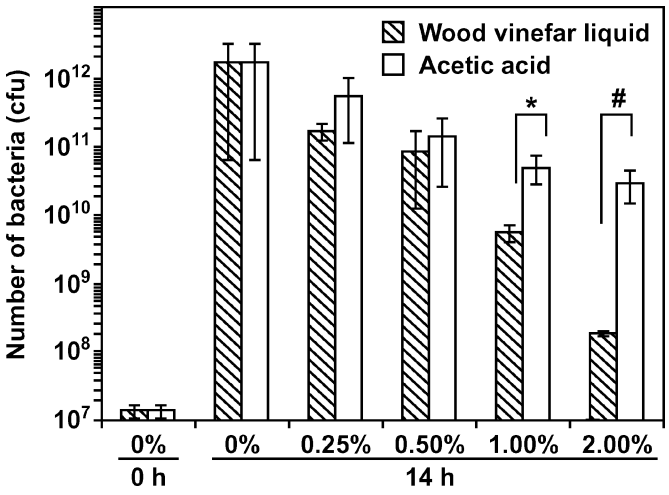

FIGURE 2. Effect of wood vinegar liquid on the growth of *Salmonella enterica* serovar Enteritidis. The *S.* Enteritidis ($1.32 \pm 0.28 \times 10^7$ cells) was incubated with 10 mL of heart infusion broth containing 0, 0.25, 0.5, 1.0, or 2.0% wood vinegar liquid or acetic acid (pH 7.0) at 37°C for 14 h, and then the number of bacteria was determined as described in the materials and methods section. Data are expressed as means ± SE of triplicate experiments. *$P < 0.026$ and #$P < 0.027$ between wood vinegar liquid and acetic acid.

homogenates (liver, spleen) or intestinal contents (duodenum, small intestine, cecum and rectum) were transferred to Eppendorf tubes. These were centrifuged at 18,000 × g for 10 min in a high-speed microcentrifuge,[9] and the resulting supernatant was discarded. Total DNA was extracted using a SepaGene kit.[10] A pair of primers was used that are specific for *S.* Enteritidis based on the *Salmonella* plasmid virulence A (*spvA*) gene of *S.* Enteritidis (5′-GCAGACATTATCAGTCTTCAGG-3′ and 5′-TCAGGTTCGTGCCATTGTCAA-3′). Ten-microliter aliquots of the PCR products were analyzed with 2% agarose gel electrophoresis.

Statistical Analysis

Student's *t*-test was performed for statistical evaluation of the results. Results are expressed as the arithmetic mean with the standard error of the mean (mean ± SE).

RESULTS

Adsorption Effect of Activated Charcoal on Bacteria

The effect of activated charcoal from the bark of an evergreen oak on bacterial adsorption was examined. The experiment was carried out using *S.* Enteritidis and *E. faecium* (i.e., intestinal bacteria). The results are shown in Figure 1. Activated charcoal caused a dose-dependent adsorption against *S.* Enteritidis and *E. faecium*. In this experiment, however, the binding capacity of activated charcoal to *E. faecium* was lower than that to the *S.* Enteritidis strain ($P < 0.031$ at 1 mg; $P < 0.0001$ at 3 mg; $P < 0.023$ at 5 mg; $P < 0.003$ at 10 mg). These results suggest that the activated charcoal oak could be effective as an adsorbent of *S.* Enteritidis and also that it may be beneficial to eliminate *S.* Enteritidis.

Effect of Wood Vinegar Liquid on the Growth of Bacteria

To elucidate the effect of wood vinegar liquid on bacterial growth, an experiment was carried out with *S.* Enteritidis, *E. faecium*, and *B. thermophilum*. When the effects of wood vinegar liquid on growth of *S.* Enteritidis were studied (Figure 2), bacterial growth was inhibited by addition of wood vinegar liquid in a dose-dependent manner. On the other hand, acetic acid less effective in reducing the growth of *S.* Enteritidis than wood vinegar liquid at final concentrations of 1.0% ($P < 0.026$) and 2.0% ($P < 0.027$), respectively. These results suggest that wood vinegar liquid would have efficacy in suppression of *S.* Enteritidis growth.

Moreover, the effect of wood vinegar liquid was assessed on growth of normal bacterial flora in the intestines, such as *E. faecium* and *B. thermophilum*. The results are shown in Table 1. Stimulation of growth of both bacteria was observed when wood vinegar liquid was added to the broth. This accelerating effect was dose dependent. In particular, difference occurred in the bacterial growth between the wood vinegar liquid-treated and untreated samples when both bacteria were incubated with wood vinegar liquid at final concentrations of 1.0% ($P < 0.05$ in *E. faecium*, $P < 0.02$ in *B. thermophilum*) and 2.0% ($P < 0.004$ in *E. faecium*, $P < 0.02$ in *B. thermophilum*). These results

[9]Tomy Seiko Co., Tokyo.
[10]Sanko Co., Tokyo.

TABLE 1. The effect of wood vinegar liquid (WVL) from the bark of an evergreen oak on the growth of bacteria[1]

Bacteria	Treatment	Bacteria (cfu) 0 h	Bacteria (cfu) 14 h
Enterococcus faecium	Untreated	$8.25 \pm 1.75 \times 10^{6}$	$5.90 \pm 1.75 \times 10^{10}$
	0.25% WVL	$8.25 \pm 1.75 \times 10^{6}$	$8.05 \pm 3.25 \times 10^{10}$
	0.50% WVL	$8.25 \pm 1.75 \times 10^{6}$	$8.30 \pm 3.80 \times 10^{10}$
	1.00% WVL	$8.25 \pm 1.75 \times 10^{6}$	$9.60 \pm 0.14 \times 10^{10a}$
	2.00% WVL	$8.25 \pm 1.75 \times 10^{6}$	$1.38 \pm 0.13 \times 10^{11b}$
Bifidobacterium thermophilum	Untreated	$6.77 \pm 3.10 \times 10^{6}$	$7.52 \pm 0.96 \times 10^{9}$
	0.25% WVL	$6.77 \pm 3.10 \times 10^{6}$	$1.15 \pm 0.64 \times 10^{10}$
	0.50% WVL	$6.77 \pm 3.10 \times 10^{6}$	$1.43 \pm 0.96 \times 10^{10}$
	1.00% WVL	$6.77 \pm 3.10 \times 10^{6}$	$2.30 \pm 0.69 \times 10^{10c}$
	2.00% WVL	$6.77 \pm 3.10 \times 10^{6}$	$3.50 \pm 1.23 \times 10^{10d}$

[a]$P < 0.05$ when compared with the untreated control.
[b]$P < 0.004$ when compared with the untreated control.
[c]$P < 0.02$ when compared with the untreated control.
[d]$P < 0.02$ when compared with the untreated control.
[1]Results are given as mean colony-forming units of bacteria ± SE.

suggest that the wood vinegar liquid would have 2 effects against intestinal bacteria: one would be an inhibitory effect on the growth of pathogenic bacteria, such as *S.* Enteritidis, the other would be a stimulatory effect on the growth of normal bacterial flora, such as *E. faecium* and *B. thermophilum*, in the intestines.

Effect of Nekka-Rich Feeding on Challenge with S. Enteritidis in Chickens

To elucidate the effect of Nekka-Rich on suppression of *S.* Enteritidis excretion in feces, chickens given Nekka-Rich were challenged orally with 1×10^{7} cfu of *S.* Enteritidis, and the fecal *S.* Enteritidis was monitored on d 2, 5, 10, and 15 after challenge. Figure 3 shows the number of *S.* Enteritidis isolated from fecal samples after challenge with the bacteria. The numbers of bacteria in untreated birds (group A) and vaccine-immunized birds (group B) decreased gradually. Although the bacteria number in feces for vaccine-immunized chickens was lower than for untreated control chickens at 2 ($P < 0.0001$) and 15 d ($P < 0.003$) after challenge, immunization of chickens with vaccine did not fully inhibit bacterial growth. On the other hand, there was a decrease in bacterial isolation between the group C and other groups at 2 d ($P < 0.0001$ compared with group A), 5 d ($P < 0.0001$ compared with group A; $P < 0.0014$ compared with group B), 10 d ($P < 0.0007$ compared with group A; $P < 0.0045$ comparing with group B), and 15 d ($P < 0.0001$ compared with group A; $P < 0.0019$ compared with group B) after challenge. On 15 d after challenge, *S.* Enteritidis was not isolated from fecal samples of group C.

Table 2 shows the direct recovery of *S.* Enteritidis from intestinal contents 16 d after the challenge. No grossly visible pathological lesions were observed at necropsy in 3 groups. In this experiment, *S.* Enteritidis was recovered from the cecal and rectal regions of chickens in the untreated control group and in the group immunized with vaccine. However, counts of *S.* Enteritidis recovered from the cecum and rectum of vaccine-immunized chickens were significantly lower than those from untreated control chickens ($P < 0.0012$ in the cecum, $P < 0.0005$ in the rectum). On the other hand, no *S.* Enteritidis was isolated from the cecum and rectum of Nekka-Rich-fed chickens in contrast to the vaccine-immunized and untreated chickens. These differences were significant ($P < 0.016$ to 0.0001). No *S.* Enteritidis was isolated from the duodenum or small intestine or other visceral organs, such as liver and spleen, in 3 groups (data not shown).

Figure 4 shows the amplification of *S.* Enteritidis-specific DNA in the cecum and rectum of untreated chickens

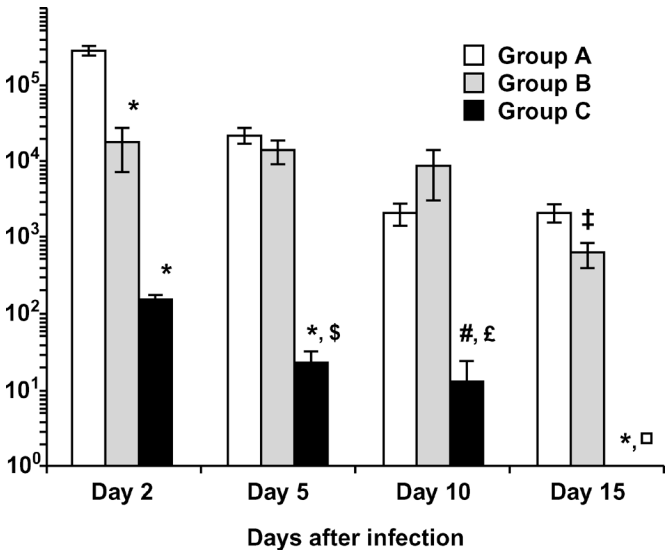

FIGURE 3. The effect of Nekka-Rich on the infection of *Salmonella enterica* serovar Enteritidis. Group A, untreated control chickens; group B, vaccine-immunized chickens; group C, chickens fed Nekka-Rich. All groups were orally challenged with 1×10^{7} cfu of *S.* Enteritidis. On d 2, 5, 10, and 15 after the challenge, the number of *S.* Enteritidis was determined as described in the materials and methods section. Data are expressed as mean colony-forming units per gram of feces from 4 chickens. *$P < 0.0001$, #$P < 0.0007$, and ‡$P < 0.003$ are statistically significant difference compared with group A; $^{\$}P < 0.0014$, $^{£}P < 0.0045$, and □$P < 0.0019$ are statistically significant difference compared with group B.

TABLE 2. Isolation of *Salmonella enterica* serovar Enteritidis from the intestinal tract after challenge with *S.* Enteritidis[1]

Treatment and group	Bacterial isolation from			
	Duodenum	Small intestine	Cecum	Rectum
Untreated (group A)	ND	ND	270 ± 39	112 ± 21
Vaccine-immunized (group B)	ND	ND	83 ± 24[b]	13 ± 5[c]
Fed Nekka-Rich (group C)	ND	ND	ND[de]	ND[df]

[b]$P < 0.0012$ when compared with the untreated chicken (group A).
[c]$P < 0.0005$ when compared with the untreated chicken (group A).
[d]$P < 0.0001$ when compared with the untreated chicken (group A).
[e]$P < 0.0036$ when compared with the untreated chicken (group B).
[f]$P < 0.016$ when compared with the untreated chicken (group B).
[1]Results are given as mean colony-forming units per gram ± SE of *S.* Enteritidis isolated from intestinal contents of 4 chickens 16 d after challenge with 1×10^7 cfu bacteria. ND = not detected.

16 d after challenge, generating a characteristic 351-bp fragment. In Nekka-Rich-fed chickens, DNA was not amplified in the cecum and rectum. No specific DNA band was amplified in DNA samples from the duodenum, small intestine, liver, or spleen (data not shown). These results are in accordance with those from bacterial isolation.

DISCUSSION

An activated charcoal has a high adsorptive capacity although it tends to be nonselective (Wicks et al., 1980; Chandy and Sharma, 1998). However, adsorptive capacity of activated charcoal depends on the pore size of activated charcoal. For removal of relatively large materials activated charcoal with large pores is needed, whereas small substances need small pores (Chandy and Sharma, 1998). In our study, *S.* Enteritidis was more effectively adsorbed by activated charcoal from the bark of an evergreen oak than *E. faecium*. The probable reason why activated charcoal has high binding capacity to *S.* Enteritidis and low binding capacity to *E. faecium* is that the activated charcoal from the bark of an evergreen oak used in this study had pores of sufficient diameter for *S.* Enteritidis but not for *E. faecium*. However, differing effectiveness of the activated charcoal for removal of *S.* Enteritidis and resident bacteria of the intestinal tract, such as *E. faecium*, has important implications for clinical application of this activated charcoal. This finding suggests that activated charcoal from the bark given orally could be able to function as an agent for reducing intestinal *S.* Enteritidis carriage and to minimize the removal of normal bacterial flora in the intestinal tract.

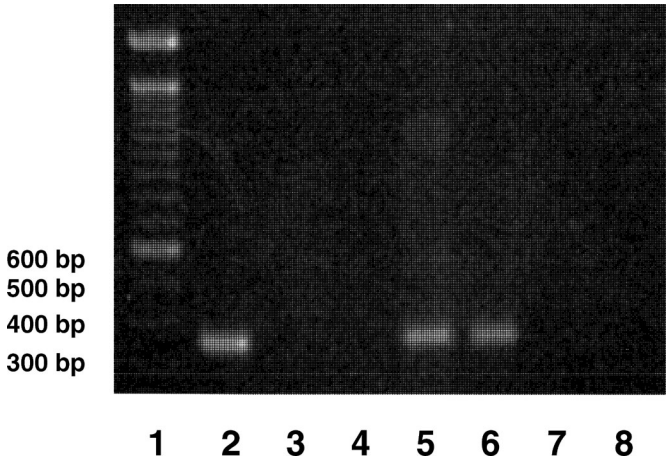

FIGURE 4. Agarose gel electrophoresis of PCR products from the intestinal contents of chickens 16 d after challenge. Lane 1, 100-bp DNA ladder marker; lane 2, *Salmonella enterica* serovar Enteritidis plasmid DNA; lanes 3 to 6, duodenum, small intestine, cecum and rectum of untreated control chickens, respectively; lanes 7 and 8, cecum and rectum, respectively, of chickens fed Nekka-Rich.

In this study, we evaluated the effect of wood vinegar liquid against the growth of some microorganisms. The growth of *S.* Enteritidis was inhibited in broth containing wood vinegar liquid (pH 7.0) but not fully in broth containing acetic acid (pH 7.0). In addition, wood vinegar liquid had growth-stimulating activity against normal bacterial flora, such as *E. faecium* and *B. thermophilum*. These results suggest that wood vinegar liquid could have properties responsible for antimicrobial activity against pathogenic bacteria, such as *S.* Enteritidis, and for growth stimulatory activity for normal bacterial flora in the intestines, such as *E. faecium* and *B. thermophilum*. However, further studies are required to verify these properties.

In the present study, significantly lower numbers of *S.* Enteritidis were observed in feces and intestinal tracts (i.e., cecum and rectum) of vaccine-immunized chickens than in untreated chickens. However, fecal shedding and intestinal colonization of *S.* Enteritidis were not completely inhibited by vaccination. This result indicates that *S.* Enteritidis vaccine was not able to induce sufficient immune responses for prevention of *S.* Enteritidis infection and confirmed results from an earlier study (Mastroeni and Ménager, 2003) in which low efficacy of vaccine for the prevention of *Salmonella* infections was reported. Comparison of bacteria isolated from the intestinal tract and feces of untreated, vaccine-immunized, and Nekka-Rich-fed chickens challenged with *S.* Enteritidis showed that colonization and excretion of bacteria were significantly suppressed in Nekka-Rich-fed chickens. The number of bacteria in the feces of Nekka-Rich-fed chickens was consistently lower than in untreated and vaccine-

immunized chickens, and no isolation of bacteria was achieved at 15 d after challenge.

Bacterial colonization of the cecum and rectum was also entirely suppressed in all chickens fed Nekka-Rich. In the PCR assay, although a specific DNA fragment was amplified in samples extracted from the cecum and rectum of untreated chickens, this fragment was not amplified in the cecum and rectum of Nekka-Rich-fed chickens. These results indicate that Nekka-Rich has a protective effect against *S.* Enteritidis infection in chickens. It has been shown that probiotics, microbes that beneficially affect the host by improving its intestinal microbial balance, such as bifidobacteria and lactic acid bacteria, are useful in treating and preventing various intestinal infections and diarrhea caused by pathogenic bacteria (Gorbach, 1990; Abe et al., 1995; Bernet-Camard et al., 1997; Bomba et al., 1999; Naidu et al., 1999; Heyman, 2000; Vold et al., 2000). In this study we showed that the wood vinegar liquid, which is contained in Nekka-Rich, has a stimulatory effect on the growth of *E. faecium*, and *B. thermophilum* acts as a probiotic (Abe et al., 1995) and also that it has the inhibitory effect against the growth of *S.* Enteritidis. Furthermore, activated charcoal of Nekka-Rich showed effectiveness for removal of *S.* Enteritidis. Thus, the protective effect of Nekka-Rich against *S.* Enteritidis challenge could be due to the growth-inhibitory effect of wood vinegar liquid of Nekka-Rich against *S.* Enteritidis, the growth-stimulating effect of wood vinegar liquid on probiotics, and the adsorption effect of activated charcoal of Nekka-Rich on *S.* Enteritidis.

The cecum is the main site of persistent infection by *S.* Enteritidis (Berthelot et al., 1998; Cerquetti and Gherardi, 2000; Van Immerseel et al., 2002). It is, therefore, likely that feeding of Nekka-Rich would remove the risk of egg contamination from feces, because excretion of *S.* Enteritidis in feces is the main factor in contamination (Thiagarajan et al., 1996; Thorns et al., 1996; Cerquetti and Gherardi, 2000; Dibb-Fuller and Woodward, 2000).

A new method that inhibits the infection of *S.* Enteritidis in chickens would clearly be valuable. We have found that administration of Nekka-Rich effectively inhibits *S.* Enteritidis colonization of mucosal surfaces. The use of Nekka-Rich as additive would be effective, therefore, in preventing *S.* Enteritidis infection in chickens, thereby facilitating eradication of the disease.

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