Production of Menaquinones by Lactic Acid Bacteria

TAKASHI MORISHITA,[1] NATSUKO TAMURA, TAKASHI MAKINO, and SATOSHI KUDO
Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo 186-8650, Japan

ABSTRACT

Lactic acid bacteria were examined for their ability to produce quinone compounds, which may include dietary sources of menaquinones. Isoprenyl quinones in bacterial cells grown in a synthetic medium were extracted and analyzed by thin layer chromatography. *Lactococcus lactis* ssp. *cremoris* (three strains), *Lactococcus lactis* ssp. *lactis* (two strains), and *Leuconostoc lactis* were selected as high producers of quinone that synthesized more than 230 nmol of quinones/g of dried cells. The quinones were presumed to be menaquinone-7 to -10 by high performance liquid chromatography. Precise molecular weights were determined by mass spectrometry for *Lactococcus lactis* ssp. *cremoris* YIT 2011 and *Leuconostoc lactis* YIT 3001 and identified as menaquinone-8 and -9 for the former and menaquinone-9 and -10 for the latter. Those strains, when grown either in reconstituted nonfat dry milk or a soymilk medium, produced a beneficial quantity for dietary supplement (i.e., 29 to 123 μg of menaquinones/L of the fermented medium).
(**Key words:** menaquinones, vitamin K_2, lactic acid bacteria)

Abbreviation key: MK = menaquinone(s).

INTRODUCTION

Vitamin K is an essential cofactor for the formation of γ-carboxyglutamic acid (Gla) residues in proteins (18, 22). The Gla-containing proteins bind calcium ions and influence, for example, blood coagulation and tissue calcification (e.g., osteocalcin found in bone tissues) (9, 19). Vitamin K deficiency has been implicated in several clinical ailments such as intracranial hemorrhage in newborn infants (8, 21) and possible bone fracture resulting from osteoporosis (15).

Vitamin K occurs naturally in two forms, namely, K1 (phylloquinone) in green plants and K2 [menaquinones (**MK**)] in animals and some bacteria (2, 4, 20, 23, 24), including intestinal bacteria (4, 20). The MK are constituents of the bacterial plasma membrane and function as redox reagents in electron transport and oxidative phosphorylation systems (20, 23).

Lactic acid bacteria have been used as starter cultures to manufacture various foods and can be generally recognized as safe (GRAS), and a qualitative study (2) has shown that some lactic acid bacteria produce MK. In many countries, the daily requirement for vitamin K is around 1 μg/kg of body weight.

An objective of the present study was to examine whether or not lactic acid bacteria produce meaningful amounts of MK, which could prevent vitamin K deficiency diseases. In the course of the investigation, lactic acid bacteria used for making fermented food products were surveyed for their ability to produce MK.

MATERIALS AND METHODS

Bacterial Strains

Bacterial strains examined in this study are listed in Table 1.

Media, Bacterial Growth, Harvest, and Lyophilization

To prepare stock cultures of lactic acid bacteria, Rogosa medium (7) was used with a minor modification. The medium comprised (per liter) D-glucose (Wako Pure Chemical Co., Osaka, Japan), 20 g; trypticase peptone (Difco Laboratories Co., Detroit, USA), 10 g; tryptose peptone (Difco Laboratories Co.), 3 g; yeast extract (Difco Laboratories Co.), 5 g; triammonium citrate, 2 g; K_2PO_4, 3 g; K_2HPO_4, 3 g; $MgSO_4 \cdot 7H_2O$, 0.575 g; $FeSO_4 \cdot 7H_2O$, 0.034 g; $MnSO_4 \cdot 4H_2O$, 12 g; L-cysteine·HCl (Wako Pure Chemicals Co.), 0.2 g; sodium acetate·$3H_2O$, 1 g; and Tween 80, 1 g. For the production of MK, the same Rogosa medium was used, except that the concentration of trypticase was increased twofold (trypticase double-strength Rogosa medium). Preliminary experiments showed that 2% or greater concentrations of trypticase were required for full growth of many strains. The Rogosa medium (7) to prepare culture stocks and test cultures for MK

Received January 8, 1999.
Accepted April 28, 1999.
[1]Corresponding author.

TABLE 1. Bacterial strains examined for production of quinone compounds.

Genus	Species	Subspecies	YIT No.[1]	Source[2]
Lactococcus	*lactis*	*cremoris*	2002	ATCC14365
Lactococcus	*lactis*	*cremoris*	2007	ATCC 19257
Lactococcus	*lactis*	*cremoris*	2011	FERM P-15769
Lactococcus	*lactis*	*cremoris*	2012	FERM P-15770
Lactococcus	*lactis*	*lactis*	2003	ATCC 11454
Lactococcus	*lactis*	*lactis*	2008	ATCC 19435
Lactococcus	*lactis*	*lactis*	2016	ATCC 13675
Lactococcus	*lactis*	*lactis*	2027	
Lactococcus	*lactis*	*lactis*	2052	NCDO 505
Lactococcus	*plantarum*		2061	ATCC 43199
Lactococcus	*raffinolactis*		2062	ATCC 43920
Leuconostoc	*lactis*		3001	ATCC 19256
Leuconostoc	*mesenteroides*	*cremoris*	3003	ATCC 19254
Leuconostoc	*mesenteroides*	*dextranicum*	3028	ATCC 19255
Enterococcus	*;faecalis*		2031	ATCC 19433
Streptococcus	*thermophilus*		2001	
Lactobacillus	*acidophilus*		0168	
Lactobacillus	*casei*			strain Shirota
Lactobacillus	*mali*		0243	ATCC 27304
Bifidobacterium	*bifidum*			strain Yakult
Bifidobacterium	*breve*			strain Yakult

[1]Registration number of the culture collections that are preserved at Yakult Central Institute for Microbiological Research, Tokyo, Japan.

[2]FERM = National Institute of Bioscience and Human Technology, Ministry of International Trade and Industry, Ibaraki, Japan; NCDO = NCFB; National Collection of Food Bacteria, Reading, United Kingdom.

production of bifidobacteria was also used, except that 10 g of D-lactose (Wako Pure Chemical Co.) and 5 g of pyruvic acid were substituted for 20 g of D-glucose and 1 g of sodium acetate·$3H_2O$. All media were adjusted to pH 6.8 with 1*N* NaOH.

Reconstituted NDM contained (per liter) 100 g of NDM, 5 g of yeast extract, 0.3 g of L-cysteine·HCl, and 2 g of $CaCl_2$. Soymilk medium comprised crude soymilk (approximately 5% crude protein; 3% crude fat; and Brix 12, pH 7.5) containing 15% D-glucose.

Lactic acid bacterial cells (ca. 10^7 cells/ml) in the Rogosa stock culture medium were inoculated into fresh Rogosa medium (for *Bifidobacterium*), trypticase double-strength Rogosa medium (for other lactic acid bacteria), reconstituted NDM, and soymilk medium. All cultures were incubated at 30°C for 48 h. Growth was assessed by measuring turbidity on a Klett-Summerson colorimeter (no. 66 filter) or a photoelectric colorimeter (for growth in the Rogosa media) and by measuring viable cell counts on a Rogosa agar medium plate (for growth in the reconstituted NDM or the soymilk medium). The agar plates were incubated at 37°C anaerobically for 3 d using gas pack anaerobic systems (Mitsubishi Gas Chemical Co., Tokyo, Japan). Cultures (ca. 3×10^8 to 1×10^9/ml) were chilled in ice water to terminate growth. For stock cultures of *Bifidobacterium*, preparation of medium, inoculation, and cultivation were performed anaerobically by using 100% nitrogen gas according to the modified Hungate method (1, 14).

Cells were harvested from the Rogosa media by centrifugation at 4000 rpm for 15 min and were washed twice with saline and lyophilized. The fermented, reconstituted NDM or soymilks were lyophilized without harvesting cells.

Extraction of Quinones from Bacterial Cells

Fifty milligrams of lyophilized cells or cultures were extracted twice with 50 ml of chloroform-methanol (2:1) for 1 h in the dark. The extracts were then centrifuged at 4000 rpm for 15 min, and the resulting supernatants were combined and evaporated to dryness. The dried residue was dissolved in 10 ml of hexane and applied on a SEP-PAK silica cartridge (Nihon Waters Co. Ltd., Tokyo, Japan). Quinones were eluted with 10 ml of hexane-diethyl ether (9:1). The eluate was evaporated to dryness under the stream of nitrogen and redissolved by adding a small amount of acetone or ethanol for the determination of quinones by TLC or HPLC.

TLC

Extracts were developed on a silica gel plate (60 F254 plate; Merck, Darmstat, Germany) with petroleum ether-diisopropyl ether (9:1) solvent (3, 6, 16). Possible quinones were quantified by UV densitometry at 254 nm (rate of flow; 0.3 to 0.4) by the gel-pattern analysis system (Doctor gel®; cooperatively developed

by the Mitani-shoji Co. Ltd., Chiba, Japan and the Yakult Honsha Co., Tokyo, Japan), which compared spots with an authentic MK-4.

HPLC

Extracts were also separated by HPLC equipped with a postcolumn electrochemical reducer (Environmental Sciences Associates, Chelmsford, MA, USA), a fluorometric detector (11, 12), and a reverse-phase column (Inertsil ODS-3; 4.6 i.d. × 250 mm; GL-Science Co. Ltd., Tokyo, Japan). An ethanol solution (92.5%) containing 0.25% $NaClO_4$ was used at a flow rate of 1.0 ml/min and 35°C. The reducer was operated at –800 mV. The excitation and emission wavelengths were 320 nm and 430 nm, respectively.

Mass Spectrometry

Menaquinones separated by HPLC were applied on a mass spectrometer (Hitachi M-80B, Hitachi Koki, Ibaraki, Japan) by a direct insertion probe at an ionization voltage of 70 eV.

RESULTS

Screening of MK-Producing Lactic Acid Bacteria

Selection of strains producing quinone. To evaluate procedures for extraction and determination of quinone compounds, two strains of Gram-positive aerobic bacteria, *Bacillus subtilis* strains YIT 6087 and YIT 6098, which are potent MK producers, were preliminarily examined with an internal standard of MK-4. As a result, 760 and 1016 nmol/g of lyophilized cells were produced, respectively.

Lactic acid bacteria were then systematically surveyed for ability to produce quinone. As shown in Table 2, many but not all of the strains displayed the ability to produce quinone compounds. Six strains produced more than 230 nmol quinones/g of lyophilized cells, and five of them were selected for further work. They were *Lactococcus lactis* ssp. *cremoris* YIT 2007, YIT 2011, and YIT 2012, *Lactococcus lactis* ssp. *lactis* YIT 2027, and *Leuconostoc lactis* YIT 3001.

Identification of MK by HPLC. Extracts from lyophilized cells of the selected strains were resolved

TABLE 2. Production of menaquinones by lactic acid bacteria as determined by TLC of extracts from lyophilized cells.

Species or subspecies	Strain[1]	Lyophilized cells/L of culture (g)	Menaquinones/g of lyophilized cells (nmol)
Lactococcus			
lactis spp. *cremoris*	YIT 2002	0.73	110
lactis ssp. *cremoris*	YIT 2007	0.56	362
lactis ssp. *cremoris*	YIT 2011	0.46	297
lactis ssp. *cremoris*	YIT 2012	0.47	600
lactis ssp. *lactis*	YIT 2003	1.00	53
lactis ssp. *lactis*	YIT 2008	0.73	150
lactis ssp. *lactis*	YIT 2016	0.21	259
lactis ssp. *lactis*	YIT 2027	0.61	230
lactis ssp. *lactis*	YIT 2052	1.00	125
plantarum	YIT 2061	1.50	29
raffinolactis	YIT 2062	1.20	30
Leuconostoc			
lactis	YIT 3001	0.77	648
mesenteroides ssp. *cremoris*	YIT 3003	1.46	44
mesenteroides ssp. *dextranicum*	YIT 3028	0.75	123
Enterococcus faecalis			
YIT 2031	0.85	194	
Lactobacillus			
acidophilus	YIT 0168	1.33	ND[2]
casei	strain Shirota	1.99	ND
mali	YIT 0243	0.68	11
Streptococcus thermophilus	YIT 2001	0.89	ND
Bifidobacterium			
bifidum	strain Yakult	0.87	ND
breve	strain Yakult	0.69	ND

[1]Registration number of the culture collections that are preserved at Yakult Central Institute for Microbial Research, Tokyo, Japan.

[2]ND = Not detected.

TABLE 3. Identification of menaquinones produced by selected strains of lactic acid bacteria as determined by HPLC of extracts from lyophilized cells.

Species or subspecies	Strain (YIT No.)[1]	Cell mass[2] (g/L)	Content of menaquinones[3] (nmol/g of lyophilized cells)				
			Total	MK-7	MK-8	MK-9	MK-10
Lactococcus lactis							
ssp. *cremoris*	2007	0.507	348	ND[4]	74	274	ND
ssp. *cremoris*	2011	0.467	534	19	135	380	ND
ssp. *cremoris*	2012	0.334	467	ND	116	307	44
ssp. *lactis*	2027	0.681	717	ND	75	392	237
Leuconostoc lactis	3001	0.603	173	7	49	117	ND

[1]Registration number of the culture collections that are preserved at Yakult Central Institute for Microbial Research, Tokyo, Japan.

[2]Cell mass was expressed as grams of lyophilized cells per liter of culture.

[3]MK = menaquinone.

[4]ND = Not detected.

into an individual isoprenologue by HPLC. The major peaks were calibrated and presumed to fit MK-7 to -10, designated as MK-n, depending on the number of isoprenyl side-chains in the molecule (Table 3). Total MK in all five strains demonstrated by HPLC was comparable to those by TLC (Table 2). A typical HPLC profile of *Leuc. lactis* YIT 3001 is shown in Figure 1.

Identification of MK with mass spectrometry. To confirm molecular structure of the presumed MK in Table 3 and Figure 1, mass spectrometry was performed for the extracts of YIT 2011 and YIT 3001. Four fractions of YIT 3001 (indicated by arrows in Figure 1) and YIT 2011, separated by HPLC, were applied to a mass spectrometer. The profiles of mass spectra provided proper molecular ions or numbers of isoprenyl units in side chains of the MK and fragment ions of m/z 225 for the naphthoquinone moiety. Consequently, the strain YIT 3001 contained mainly MK-9 [m/z 784 ($M^{+}\cdot$)] and MK-10 [m/z 852 ($M^{+}\cdot$)], and YIT 2011 contained MK-8 [m/z 716 ($M^{+}\cdot$)]) and MK-9 [m/z 814 ($M^{+}\cdot$)].

Production of MK in Reconstituted NDM or Soymilk Medium

The MK-producing lactic acid bacteria could be a source of vitamin K in various fermented products. Therefore, selected strains were grown in both a reconstituted NDM and a soymilk medium to determine whether meaningful amounts of MK could be produced. Although only two strains (YIT 2011 and YIT 2012) grew in the reconstituted NDM, all five strains

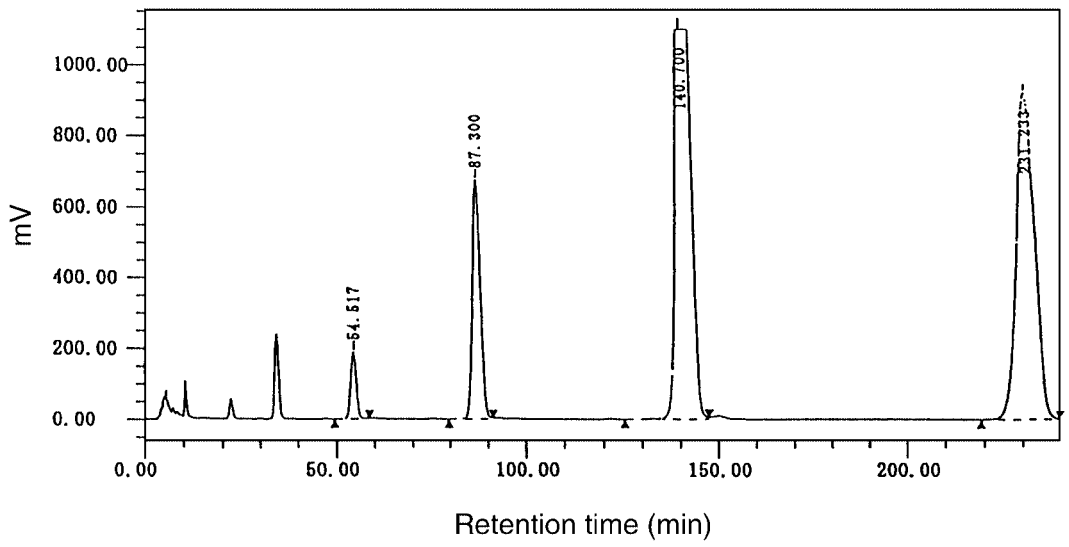

Figure 1. Profiles of menaquinones in extracts of *Leuconostoc lactis* YIT 3001 by HPLC.

TABLE 4. Production of menaquinones by *Lactococcus lactis* ssp. *cremoris* strains YIT 2011 and YIT 2012 as determined by HPLC of extracts from the lyophilized, reconstituted NDM culture.

Species or supspecies	Strain (YIT No.)[1]	Content of menaquinones[2] (nmol)									
		Total		MK-7		MK-8		MK-9		MK-10	
		/g[3]	/L[4]	/g	/L	/g	/L	/g	/L	/g	/L
Lc. lactis ssp. *cremoris*	2011	2.19	202 90[5]	0.18	17	0.87	71	1.22	113	ND[6]	ND
Lc. lactis ssp. *cremoris*	2012	0.70	65 29[5]	0.10	9	0.24	23	0.30	28	ND	ND

[1]Registration number of the culture collections that are preserved at Yakult Central Institute for Microbial Research, Tokyo, Japan.

[2]MK = menaquinone.

[3]/g = Content per gram of the fermented reconstituted NDM culture.

[4]/L = Content per liter of the fermented reconstituted NDM culture.

[5]Estimated as MK-7 (micrograms per liter).

[6]ND = Not detected.

acidified the soymilk medium. *Lactococcus lactis* ssp. *cremoris* YIT 2011 and YIT 2012 produced a considerable amount of MK in the milk medium (Table 4). The forms of MK ranged from MK-7 to -10, which is consistent with those from the Rogosa medium cultures (see Table 3). In addition, all fermented soymilk media contained quantities of MK (Table 5) comparable to that of the milk media. As in milk cultures, the forms of MK in the soymilk media ranged from MK-7 to MK-10.

DISCUSSION

We selected *Lc. lactis* ssp. *cremoris* YIT 2007, YIT 2011, YIT 2012; *Lc. lactis* ssp. *lactis* YIT 2027, YIT 2016; and *Leuc. lactis* YIT 3001 as high MK-producing

TABLE 5. Production of menaquinones by five stains of lactic acid bacteria as determined by HPLC of extracts from the lyophilized soymilk medium culture.

Species or subspecies	Strain (YIT No.)[1]	Content of menaquinones[2] (nmol)									
		Total		MK-7		MK-8		MK-9		MK-10	
		/g[3]	/L[4]	/g	/L	/g	/L	/g	/L	/g	/L
Lactococcus lactis ssp. *cremoris*	2007	0.99	122 54[5]	0.02	2	0.22	27	0.75	92	ND[6]	ND
	2011	2.58	249 110[5]	0.44	5	0.50	57	1.64	187	ND	ND
	2012	1.51	169 75[5]	0.02	2	0.34	38	1.15	129	ND	ND
Lactococcus lactis ssp. *lactis*	2027	0.75	87 39[5]	0.01	1	0.17	20	0.57	66	ND	ND
Leuconostoc lactis	3001	2.60	278 123[5]	0.1	1	0.24	27	1.63	180	0.63	70

[1]Registration number of the culture collections that are preserved at Yakult Central Institute for Microbial Research, Tokyo, Japan.

[2]MK = menaquinone.

[3]/g = Content per gram of the fermented soymilk medium culture.

[4]/L = Content per liter of the fermented soymilk medium culture.

[5]Estimated as MK-7 (micrograms).

[6]ND = Not detected.

lactic acid bacteria. To our knowledge, this paper is the first to describe quantitative determination of MK in lactic acid bacteria. The MK production of *Lc. lactis* ssp. *cremoris* YIT 2012 and *Leuc. lactis* YIT 3001 was close to that of bacilli, which was more than expected, based on the previous reports (2, 10). The major forms of MK ranged from MK-7 to -10. This result was in agreement with the previous reports describing that MK-7 to -10 distribute among many Gram-positive bacteria including *Bacillus* (24), lactic acid bacteria (2), and other anaerobic bacteria (20).

Requirements for vitamin K are still controversial despite recommendations that a daily intake of 1 μg/kg body weight is safe and adequate (5). This recommendation only relates to the blood-coagulation function of this vitamin. In addition, the insistent belief that intestinal bacteria are an important source of vitamin K was critically reviewed and found to be erroneous by Lipsky (17). A rational criterion for vitamin K is that all vitamin K-dependent proteins should be in fully γ-carboxylated form. In this context, the blood coagulation time was not significantly affected by intake of dietary vitamin K (8); some parameters of bone metabolism, however, are changeable by vitamin K administration. For example, reduced amounts of hydroxyproline and increased amounts of osteocalcin (γ-carboxylated protein) in urine caused by a 1-mg administration of vitamin K to postmenopausal women were observed by Knapen et al. (15). In addition, MK-7 concentrations in blood were lower in elderly Japanese women with bone fractures than in those without, as reported by Hosoi (13). A source of MK-7 is attributed to natto, which is a traditional and popular Japanese food made of soybeans fermented by bacilli. Therefore, quantities in this study [29 to 123 μg of MK (as MK-7)/L] of the fermented products of lactic acid bacteria (Tables 4 and 5) can contribute to human health, except in people who receive warfarin anti-blood coagulating treatment.

CONCLUSIONS

Based on quantitative determination of MK in lactic acid bacteria, we examined five high-producing MK strains. Those strains, when grown in a reconstituted NDM or a soymilk medium produced a meaningful amount of MK (MK-7 to -10) that supplemented a vitamin K requirement for humans. Therefore, we concluded that these strains would be useful as starter cultures for dairy and other food fermentation or dietary supplements by themselves.

ACKNOWLEDGMENTS

We are deeply grateful to Masaaki Watanuki for his valuable discussion and encouragement throughout the work and to Mika Miura and Kanako Shimizu for their expert technical assistance.

REFERENCES

1 Azuma, R. 1970. Validity of transfer of the taxonomical position of *Corynebacterium pseudopyogenes* from genus *Corynebacterium* to genus *Actinomyces*. Pages 493–505 *in* Proc. First Int. Conf. Culture Collections. H. Iizaka and T. Hasegawa, ed. Univ. Tokyo Press, Tokyo, Japan.

2 Collins, M. D., and D. Jones. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication Microbiol. Rev. 45:316–354.

3 Collins, M. D., H. N. Shah, and D. E. Minnikin. 1980. A note on the separation of natural mixtures of bacterial menaquinones using reverse phase thin-layer chromatography. J. Appl. Bacteriol 48:277–282.

4 Conly, J. M., and K. Stein, 1992. The production of menaquinones (vitamin K2) by intestinal bacteria and their role in maintaining coagulation homeostasis. Prog. Food Nutr. Sci. 16:307–343.

5 Department of Health. 1991. Reports on health and social subjects no 41: dietary reference values for food energy and nutrients for the United Kingdom. Her Majesty's Stationery Office (HMSO), London, United Kingdom.

6 Dunphy, P. J., and A. F. Brodie. 1971. The structure and function of quinones in respiratory metabolism. Methods Enzymol. 18 (Part C):407–461.

7 Efthymiou, C., and P. A. Hansen. 1962. An antigenic analysis of *Lactobacillus acidophilus*. J. Infect. Dis. 110:258–267.

8 Ferland, G., J. A. Sadowski, and M. E. O'Brien. 1993. Dietary induced subclinical vitamin K deficiency in normal human subjects. J. Clin. Invest. 91:1761–1768.

9 Hauschka, P. V., and M. L. Reid. 1978. Vitamin K dependence of a calcium-binding protein containing gamma-carboxyglutamic acid in chicken bone. J. Biol. Chem. 253:9063–9068.

10 Hess, A., R. Hollander, and W. Mannheim. 1979. Lipoquinones of some spore-forming rods, lactic-acid bacteria and Actinomycetes. J. Gen. Microbiol. 115:247–252.

11 Hirauchi, K., T. Sakano, and A. Morimoto. 1986. Measurement of K vitamins in humans and animal plasma by high-performance liquid chromatography with fluorometric detection. Chem. Pharm. Bull. 34:845–849.

12 Hirauchi, K., T. Sakano, S. Notsumoto, T. Nagaoka, A. Morimoto, K. Fujimoto, S. Masuda, and Y. Suzuki. 1991. Assay methods for K vitamins in biological materials by high-performance liquid chromatography with fluorometric determination. Vitamins (Jpn.) 65:13–21.

13 Hosoi, T. 1996. Recent progress in treatment of osteoporosis. Nippon Ronen Igakkai Zasshi 33:240–244.

14 Hungate, R. E. 1966. A roll-tube method for cultivation of strict anaerobes. Pages 117–132 *in* Methods in Microbiology. Vol. 3B. J. R. Norris and D. W. Ribbons, ed. Academic Press, Inc., New York, NY.

15 Knapen, M. H., K. Hamulyák, and C. Vermeer. 1989. The effect of vitamin K supplementation on circulation osteocalcin (bone Gla protein) and urinary calcium excretion. Ann. Int. Med. 111:1001–1005.

16 Lichtenthaler, H. K., and K. Borner. 1982. Separation of prenylquinones, prenyl vitamins and prenols on thin-layer plates impregnated with silver nitrate. J. Chromatogr. 242:196–201.

17 Lipsky, J. J. 1994. Nutritional sources of vitamin K. Mayo Clin. Proc. 69:462–466.

18 Olson, R. E. 1984. The function and metabolism of vitamin K. Annu. Rev. Nutr. 4:281–337.

19 Price, P. A., A. S. Otsuka, J. W. Poser, J. Kristaponis, and N. Raman. 1976. Characterization of a gamma-carboxyglutamic acid-containing protein from bone. Proc. Natl. Acad. Sci. USA 73:1447–1451.

20 Ramotar, K., J. M. Conly, H. Chubb, and T. J. Louie. 1984. Production of menaquinones by intestinal anaerobes. J. Infect. Dis. 150:213–218.
21 Shearer, M. J. 1995. Vitamin K. Lancet 345:229–234.
22 Suttie, J. W. 1985. Vitamin K-dependent carboxylase. Annu. Rev. Biochem. 54:459–477.
23 Taber, H. 1980. Functions of vitamin K2 in microorganisms. Pages 177–187 *in* Vitamin K metabolism and vitamin K-dependent proteins. J. W. Suttie, ed. Univ. Park Press, Baltimore, MD.
24 Watanuki, M., and K. Aida. 1972. Significance of quinones in the classification of bacteria. J. Gen. Appl. Microbiol. 18:469–472.