SHORT COMMUNICATION

Lipoquinones of some Spore-forming Rods, Lactic-acid Bacteria and Actinomycetes

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The respiratory quinones of 73 strains of Gram-positive bacteria including spore-forming rods, lactic-acid bacteria and actinomycetes were examined. Menaquinones with seven isoprenoid units (MK-7) were the main quinone type found in representatives of the genus *Bacillus* and in *Sporolactobacillus inulinus*. However, a strain of *B. thuringiensis* produced MK-8 in addition to MK-7, and strains of *B. lentus* and *B. pantothenicus* appeared to produce MK-9 and MK-8, respectively, with no MK-7. In the clostridia and lactic-acid bacteria, no quinones were found, except in *Pediococcus cerevisiae* NCTC 8066 and *Lactobacillus casei* subsp. *rhannosus* ATCC 7469, which contained menaquinones, and *Streptococcus faecalis* NCTC 775 and HIM 478-1, which contained demethylmenaquinones, in relatively low concentrations. Menaquinones were also found in the actinomycetes (except *Actinomyces odontolyticus* and *Bifidobacterium bifidum* which did not produce any quinones) and in *Protaminobacter alboflavus* ATCC 8458, the so-called *Actinobacillus actinoides* ATCC 15900 and *Noguchia granulosis* NCTC 10559.

INTRODUCTION

There is now good evidence that respiratory quinones can be very useful in the classification of bacteria. As a rule, members of the family Enterobacteriaceae contain ubiquinones (Q), menaquinones (MK) and/or demethylmenaquinones (DMK) (Bishop *et al.*, 1962; Whistance *et al.*, 1969), whereas those of the taxa *Actinobacillus*, *Haemophilus* and *Pasteurella* and many Vibrionaceae produce Q and/or DMK (Holländer & Mannheim, 1975; Callies & Mannheim, 1978; Mannheim *et al.*, 1978); the majority of the strictly aerobic, Gram-negative chemo-organotrophs produce only Q with the exception of the cytophagas and myxobacters which appear, like the anaerobic Gram-negative bacteria, to produce only MK (Bishop *et al.*, 1962; Lester & Crane, 1959; Whistance *et al.*, 1969; Yamada *et al.*, 1969; Callies & Mannheim, 1978). Members of the Gram-positive taxa synthesize either MK or DMK (*Streptococcus faecalis*) or do not produce quinones (Jeffries *et al.*, 1969; Watanuki & Aida, 1972; Yamada *et al.*, 1976a, b, 1977a, b; Holländer *et al.*, 1977; Collins *et al.*, 1977, 1979a, b; Minnikin *et al.*, 1978); some misclassified strains contain Q and should no longer be classified with Gram-positive bacteria (Collins *et al.*, 1979a; Holländer & Vobis, 1979).

In the present study the lipoquinones of some Gram-positive bacteria, particularly spore-forming rods, lactic-acid bacteria, and some actinomycetes, were examined for the type, amounts, and prevalent isoprenologues of the quinones they contained.

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Table 1. *Quinone systems in Gram-positive bacteria*

Species	Strain	Source*	Growth conditions,† temp. (°C)	Quinones‡ [µmol (g protein) ⁻¹]	No. of isoprene units in side chain§
<i>Bacillus alcalophilus</i>	Vedder	NCTC 4553	1, 37	2210 MK	7, (3)
<i>B. alvei</i>	Kocur 1581	HIM 417-1	1, 30	910 MK	7
<i>B. aminovorans</i>	Den Dooren de Jong, type	NCTC 2870	1, 30	1300 MK	7, (2)
<i>B. amyloliquefaciens</i>	Fukumoto, proposed type	DSM 7	1, 30	1660 MK	7, (2)
<i>B. aneurinololyticus</i>	Kimura	ATCC 12856	1, 30	860 MK	7
<i>B. anthracis</i>	Henry 110, type	HIM 354-1	1, 30	+ MK	NT
<i>B. badius</i>	Proom, CN 2918	CCM 2113	1, 30	1660 MK	7, (3)
<i>B. brevis</i>	Lederle Labs. 5	CCM 1613	1, 30	1350 MK	7
<i>B. cereus</i>	Proom, CN 2721	HIM 455-8	1, 30	540 MK	7
<i>B. circulans</i>	ZoBell 563	CCM 1611	1, 30	480 MK	7
<i>B. cirriferagellosus</i>	Smith 784	ATCC 14411	16, 30	1840 MK	NT
<i>B. coagulans</i>	ZoBell 567	CCM 1082	1, 30	1530 MK	7, (3)
<i>B. epiphytus</i>	ZoBell 585	ATCC 14412	16, 30	1830 MK	7, (3)
<i>B. filicolonicus</i>	Proom, CN 1758	ATCC 14413	16, 30	210 MK	7
<i>B. firmus</i>	Gibson 68	CCM 37	1, 30	1200 MK	7, (3)
<i>B. freudenreichii</i>	Stokes W 25	NCTC 4823	1, 30	1460 MK	7
<i>B. globisporus</i>	Stokes W 16b, type	CCM 2119	1, 20	1620 MK	7, (3)
<i>B. insolitus</i>	Nakayama M-8, type	CCM 2175	1, 20	1550 MK	7, (3)
<i>B. laevolacticus</i>	Holst 846	DSM 422	6, 30	580 MK	7
<i>B. larvae</i>	Gordon 661	ATCC 14706	4, 30	320 MK	7
<i>B. laterosporus</i>	Gibson 165, type	CCM 831	1, 30	650 MK	7, (3)
<i>B. lentus</i>	Proom, CN 2789	ATCC 10840	1, 30	930 MK	9, (3)
<i>B. licheniformis</i>	Gordon 1264	CCM 35	1, 30	890 MK	9, (3)
<i>B. macerans</i>	Proom, CN 1013	CCM 864	1, 30	540 MK	7, (3)
<i>B. macquariensis</i>	Marshall, 673, type	CCM 1610	1, 30	720 MK	7, (3)
<i>B. maroccanus</i>	Delaporte 6719, type	NCTC 10419	3, 20	420 MK	7, (3)
<i>B. medusa</i>	Delaporte, type	ATCC 25099	1, 30	650 MK	7, (3)
<i>B. megaterium</i>	Ivánovics	ATCC 25621	1, 37	1090 MK	7, (3)
<i>B. pantothenicus</i>	Proom, CN 3038, type	CCM 1711	1, 30	316 MK	7
<i>B. pasteurii</i>	Gibson 22, prop type	NCTC 8162	1, 30	1160 MK	8, (2)
<i>B. polymyxa</i>	NRC C67	NCTC 4822	17, 30	730 MK	7, (2)
<i>B. popilliae</i>	Tashiro	CCM 2000	1, 30	790 MK	7
<i>B. psychrophilus</i>	Stokes W16a, type	ATCC 14706	4, 30	320 MK	7
<i>B. psychrosaccharolyticus</i>	Stokes, type	CCM 2117	1, 20	1550 MK	7, (3)
<i>B. pulvifaciens</i>	Smith 1284, type	CCM 2118	1, 20	320 MK	7, (3)
<i>B. pumilus</i>	Smith 735	CCM 38	1, 30	650 MK	7, (2)
<i>B. racemilacticus</i>	Nakayama M-14, type	CCM 1726	1, 30	1450 MK	7
<i>B. sphaericus</i>	Smith 344	DSM 444	1, 30	110 MK	7
<i>B. stearothermophilus</i>	Oxoid	CCM 2176	1, 30	1740 MK	7, (3)
<i>B. subtilis</i>	Smith 231	HIM 432-2	1, 60	470 MK	7
		ATCC 6633	1, 30	750 MK	7

Table 1 (continued)

Species	Strain	Source*	Growth conditions, † temp. (°C)	Quinones ‡ [nmol (g protein) ⁻¹]	No. of isoprene units in side chain§
<i>B. thiaminolyticus</i>	Arima	ATCC 11376	1, 30	860 MK	7, (2)
<i>B. thuringiensis</i>	Steinhaus, suggested working type	CCM 19	1, 30	1190 MK	8, 7, (3)
<i>Sporolactobacillus inulinus</i>	Suzuki, type	ATCC 15538	18, 37	3300 MK	7
<i>Clostridium butyricum</i>	Howie, suggested working type	NCTC 7423	2, 37	ND	
<i>C. chauvoei</i>	Biovetta 208	CCM 5418	2, 37	ND	
<i>C. histolyticum</i>	Weinberg	CCM 5943	2, 37	ND	
<i>C. oedematiens</i>	Jolly	CCM 5949	2, 37	ND	
<i>C. paraputrificum</i>	Jena	Schering 24	2, 37	ND	
<i>S. Streptococcus</i> sp.	77007/71	HIM 411-5	3, 37	ND	
<i>S. faecalis</i>	Tissier, suggested working type	NCTC 775	3, 37	314 DMK	NT
<i>S. faecalis</i>	67264/68	HIM 478-1	3, 37	33 DMK	NT
<i>S. pyogenes</i>		HIM 422-1	3, 37	ND	
<i>Leuconostoc cremoris</i>	SMR 68	DSM 20200	8, 30	ND	
<i>L. paramesenteroides</i>	Holzapel Lc2	DSM 20186	8, 30	ND	
<i>Pediococcus cerevisiae</i>	Pederson F 166, suggested working type	NCTC 8066	11, 30	6 MK	NT
<i>Aerococcus viridans</i>	54929/64	HIM 35-6	15, 37	ND	
<i>Gemella haemolyans</i>	Berger H3	NCTC 10243	8, 37	ND	
<i>Lactobacillus acidophilus</i>	Reitger 4B	NCTC 1723	8, 37	ND	
<i>L. brevis</i>	Orla-Jensen 14, type	DSM 20054	8, 37	ND	
<i>L. casei</i> subsp. <i>rhamnosus</i>	Strong, type	ATCC 7469	8, 37	60 MK	NT
<i>L. odontolyticus</i>	McIntosh Type 1	NCTC 1406	8, 37	ND	
<i>Brochothrix thermosphacta</i> (<i>Microbacterium thermosphactum</i>)	Sulzbacher SW 26	DSM 20171	7, 37	320 MK	9
<i>Actinomyces bovis</i>	Carne D 121	NCTC 4500	10, 37	10 MK	NT
<i>A. odontolyticus</i>	Batty A10	NCTC 9935	10, 37	ND	
<i>Bifidobacterium bifidum</i>	Hayward HA	NCTC 10471	13, 37	ND	
<i>Bacterionema matruchotii</i>	Gilmour, type	NCTC 10245	12, 37	400 MK	NT
<i>Mycobacterium smegmatis</i>	SN41	HIM 358-2	3, 30	4250 MK	9, 8
<i>Dermatophilus congolensis</i> (<i>D. dermatonomus</i>)	Selbie SFR	NCTC 7915	7, 37	150 MK	9
<i>Thermoactinomyces sacchari</i>	Lacey A 978, type	NCTC 10721	14, 50	890 MK	NT
<i>Protaminobacter alboblavus</i>	den Dooren de Jong	ATCC 8458	3, 22	760 MK	NT
<i>Actinobacillus actinoides</i>	Bucsav 61.6	ATCC 15900	3, 22	141 MK	NT
<i>Noguchia granulosis</i>	Reiner-Deutsch	NCTC 10559	3, 30	549 MK	NT

* ATCC, American Type Culture Collection, Rockville, U.S.A.; NCTC, National Collection of Type Cultures, London; HIM, Culture Collection of the Hygiene-Institute, Marburg, F.R.G.; DSM, German Collection of Microorganisms, München, F.R.G.; CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia.

† See Methods.

‡ MK, Menquinone; DMK, demethylmenquinone; ND, no lipoquinones detected at ≥ 1 nmol (g protein)⁻¹.

§ Numbers of isoprene units in parentheses were estimated according to R_F values (Dunphy *et al.*, 1971); NT, not tested.

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METHODS

Strains. The organisms studied are listed in Table 1. Cultures were kept in the lyophilized state under vacuum at room temperature and their identity was checked using conventional taxonomic criteria before use.

Growth conditions. The test strains were examined under the conditions cited in Table 1 and below, using the following media: 1, Tryptic Soy Broth (Difco), aerobic culture; 2, Proteose Peptone No. 3 (Difco) (2%, w/v) plus NaCl (0.5%, w/v), pH 7.2, anaerobic culture; 3, Proteose Peptone No. 3 (Difco), aerobic; 4, medium 20 (ATCC, 1974), aerobic; 5, medium 573 (ATCC, 1974), aerobic; 6, medium 435 (ATCC, 1974), aerobic; 7, Brain-Heart Infusion (Difco), aerobic; 8, medium 11 (DSM, 1977), aerobic; 9, medium 19 (ATCC, 1974), aerobic; 10, *Actinomyces* broth (BBL), oxygen-limited; 11, Tryptic Soy Broth plus fumarate (0.05 M), anaerobic; 12, Brain-Heart Infusion plus yeast extract (2%, w/v), oxygen-limited; 13, medium 58 (DSM, 1977), anaerobic; 14, medium 635 (ATCC, 1974), aerobic; 15, Proteose Peptone No. 3 (Difco) plus bovine serum (1%, v/v), aerobic; 16, Tryptic Soy Broth plus NaCl (3%, w/v), aerobic; 17, Tryptic Soy Broth plus urea (5%, w/v), aerobic; 18, medium 149 (ATCC, 1974), aerobic; 19, Proteose Peptone No. 3 (Difco) plus glucose (0.01%, w/v) plus NaHCO_3 (0.01 M), aerobic; 20, Proteose Peptone No. 3 (Difco) plus fumarate (0.02 M), oxygen-limited; 21, Tryptic Soy Agar (Difco), anaerobic. The bacteria were cultivated and harvested as described elsewhere (Holländer & Mannheim, 1975).

Extraction and determination of quinones and protein. The quinones were extracted as previously described (Holländer *et al.*, 1977) and their isoprenologues were determined by reversed-phase thin-layer chromatography on kieselgel 60 (Merck, Darmstadt, F.R.G.) impregnated with paraffin according to Dunphy *et al.* (1971). Standards of MK-6, MK-7, MK-8, MK-9 and MK-10 were generously supplied by Hoffmann-La Roche, Basel, Switzerland.

RESULTS AND DISCUSSION

The results (Table 1) confirm those of earlier studies which showed that Gram-positive bacteria synthesize only naphthoquinones or lack all quinones (Jeffries *et al.*, 1969; Watanuki & Aida, 1972; Yamada *et al.*, 1976*a, b*, 1977*a, b*; Holländer *et al.*, 1977; Collins *et al.*, 1977, 1979*a, b*; Minnikin *et al.*, 1978).

On the basis of quinone distribution the test strains could be divided into three main categories: (i) those with a quinone content ≥ 140 nmol (g protein) $^{-1}$ [*Actinobacillus actinoides*, the *Bacillus* species investigated, *Bacterionema matruchotii*, *Dermatophilus congolensis*, *Brochothrix thermosphacta*, *Mycobacterium smegmatis*, *Noguchia granulosis*, *Protaminobacter albobiflavus*, *Sporolactobacillus inulinus*, *Streptococcus faecalis* (strain Tissier synthesizes DMK) and *Thermoactinomyces sacchari*]; (ii) those with a quinone content ≤ 140 nmol (g protein) $^{-1}$ [*Lactobacillus casei* subsp. *rhamnosus*, *Pediococcus cerevisiae* and *Streptococcus faecalis* (strain HIM 478-1 synthesizes DMK)]; and (iii) those with no quinones [*Actinomyces odontolyticus*, *Bifidobacterium bifidum*, the *Clostridium* species investigated, *Gemella haemolysans*, *Lactobacillus* species (except ATCC 7469), *Leuconostoc* species and *Streptococcus* species (except *S. faecalis*)].

The quinone contents of the organisms listed in category (i) are capable of sustaining an energetically useful electron transport process; e.g. for *Haemophilus parainfluenzae* 1 Fleming, the minimum quinone content which mediates an effective electron transport system was in the order of 0.01 μmol (g protein) $^{-1}$ (Holländer, 1976). Similar results have been obtained with *Acinetobacter* species and *Proteus mirabilis* (R. Holländer, unpublished results). The observation that strains received as *Actinobacillus actinoides* and *Noguchia granulosis* contain MK is in accordance with their positive Gram-stain reaction, their growth characteristics and some of their physiological properties, suggesting that they are misclassified and may belong to the order Actinomycetales (W. Mannheim, unpublished results). The functional significance of the quinones of the strains in category (ii), which are in low concentrations, may be respiratory as proposed by Dolin & Baum (1965) for *Streptococcus faecalis* (cf. Holländer *et al.*, 1977). This view, however, has not so far been substantiated by the analysis of electron transport sequences and energy yields.

The absence of quinones in the clostridia and lactic-acid bacteria tested (category iii) is consistent with the lack of a respiratory chain in the former (cf. Smith & Hobbs, 1974) and of cytochromes in the latter (cf. Rogosa, 1974). The current classification of *Gemella haemo-*

lysans with the Streptococcaceae (Reyn, 1974) is supported by the lack of quinones in the *Gemella* strain investigated since Gram-negative bacteria as far as is known are capable of producing quinones.

Recent publications on quinones as taxonomically useful characters have shown the importance of the length and the hydrogenation of the side chain (Yamada *et al.*, 1976*a, b*, 1977*a, b*; Collins *et al.*, 1978). Using reversed-phase chromatography, the R_F values of quinones with hydrogenated side chains are greater than those of quinones with the same number of isoprene units but fully unsaturated side chains. In the present study only reversed-phase chromatography and fully unsaturated standard menaquinones were used. Undoubtedly, the data given for the estimated numbers of isoprene units of menaquinone side chains (Table 1) would be impaired by partial hydrogenation which may occur (Dunphy *et al.*, 1971). Considering this restriction, the number of the isoprene units in the predominant menaquinone of strains classified in the genus *Bacillus* appears to be constant despite the large phylogenetic distances between the individual members of this taxon; bacilli range in DNA base composition from 35 to 52 mol% guanine plus cytosine (Baptist *et al.*, 1978). MK-7 was the main quinone type in *Bacillus* strains though smaller amounts of MK-3 or MK-2 were also found. However, the major quinone of *B. lentus* in reversed-phase thin-layer chromatography behaved like MK-9, and that of *B. pantothenicus* like MK-8. To give an unambiguous interpretation of these findings, additional data on the degree of unsaturation of the isoprenoid chains is required.

Watanuki & Aida (1972) also reported mainly MK-7 in *Bacillus* strains, but did not find quinones with shorter side chains. The few strains of actinomycetes and related bacteria that have been investigated contain menaquinones with isoprenoid side chains of 8 to 11 units. These results are in accordance with other published data (Collins *et al.*, 1977, 1979*a*; Yamada *et al.*, 1977*a, b*; Minnikin *et al.*, 1978; cf. Minnikin & Goodfellow, 1976).

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