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Quinone Profiles of *Thermoplasma acidophilum* HO-62

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Quinones of *Thermoplasma acidophilum* HO-62 were analyzed by high-performance liquid chromatography, mass spectrometry, and nuclear magnetic resonance. Menaquinone, methionaquinone, and 2-*trans* and 2-*cis* forms of thermoplasmaquinone were identified. The relative amount of thermoplasmaquinone increased under anaerobic conditions, and those of menaquinone and methionaquinone increased under aerobic conditions.

Isoprenoid quinones are widely distributed in the bacterial plasma membrane and play important roles in the electron transport system (14). The bacterial quinone profile has often been used for microbial taxonomy (4–6, 15). The relationship between the quinone profile and growth conditions has also been discussed, since each quinone has specific redox potential and its content is expected to affect the respiratory system (7, 12, 13, 16).

Thermoplasma acidophilum is a facultative anaerobic and thermophilic archaeon. Two naphthoquinones, thermoplasmaquinone-7 (TPQ-7), which is a derivative of methylmenaquinones, and menaquinone-7 (MK-7) have been isolated from strain 122-1B2 (3, 11). A methyl group of methylmenaquinone isolated from *Alteromonas putrefaciens* was reported to be located at position 8 on the naphthoquinone ring (10). However, the exact position of the methyl group in TPQ-7 has not been reported yet. In this study, we determined the position of the methyl group. The other quinones in *T. acidophilum* HO-62 were also analyzed. We also investigated the influence of aeration on the relative amount of naphthoquinones in *T. acidophilum*.

T. acidophilum HO-62 was statically grown in 10 liters of a medium described by Yasuda et al. (18). Total lipids were extracted by the Bligh-Dyer method (2) from the harvested cells (14.8 g [wet weight] in total) and then were passed through a Sephadex G-25 column (17). The total lipid was applied on to a silica gel column (30 by 250 mm) equilibrated with chloroform. Neutral lipid was eluted with 200 ml of chloroform and subjected to high-performance liquid chromatography (HPLC). Four peaks (a to d) were observed (Fig. 1). The structures of the compounds corresponding to these peaks obtained in this study are shown in Fig. 2.

Electron impact mass spectrometry was used to analyze the compounds corresponding to the four peaks shown in Fig. 1. Both TPQ-7(a) and TPQ-7(b) showed a molecular ion $[M]^+$ at *m*/*z* 662, and fragment ions at *m*/*z* 647, 594, 526, 457, 389, 321, and 253, which are similar to those reported for TPQ with seven isoprene units (3). TPQ-7(a) and TPQ-7(b) are considered to be *cis-trans* isomers. MTK-7(c) that showed $[M]^+$ at *m*/*z* 680 seemed to be one of the methionaquinones (2-methylthio-3-multiprenyl-1, 4-naphthoquinone) (9), with seven isoprene units. The fragment ion observed at *m*/*z* 634 can be explained by the loss of $S{=}{=}CH_2$ from $[M]^+$, caused by hydrogen transfer. This fragmentation was similar to that of MTK-7(H_4) (9). The molecular ion derived from ^{34}S ($[M+2]^+$, *m*/*z* 682) was not distinct, because it overlapped with isotope peaks of ^{13}C. MK-7(d) showed $[M]^+$ at *m*/*z* 648. The fragmentation pattern was similar to that reported for MK-7 (10).

Nuclear magnetic resonance (NMR) spectra of these quinones were measured in deuterochloroform ($CDCl_3$). The ^1H NMR spectra of TPQ-7(a) and TPQ-7(b) were similar, except for the signals of b_2 and f_{1-2} in TPQ-7(a) (Fig. 3). The ^1H NMR spectrum of TPQ-7(b) agreed well with that of TPQ-7 (3). The signal of f_{1-2} in TPQ-7(a) was determined by the one-dimensional steady-state differential nuclear Overhauser effect (1D-NOE) presaturated at peak c. Because the correlation signals of f_{1-2} were observed, the double bond of an isoprene unit which was nearest to the naphthoquinone ring was thought to be a *cis* form (Fig. 4). TPQ-7(b) was found to be a *trans* form, because the 1D-NOE spectrum of TPQ-7(b) showed the correlation signal of g_1 (data not shown). A fraction of TPQ-7(a) was isomerized to TPQ-7(b) in a storage solution and vice versa (data not shown).

To investigate the position of the CH_3 group in the naphthoquinone ring of TPQ, the heteronuclear multiple-bond correlation (1) spectrum of TPQ-7(b) was measured (Fig. 5). The CH_3(d) of TPQ was found to be present at position 8 (a8). The position of the CH_3 group in TPQ-7 of *T. acidophilum* was the same as that in methylmenaquinone-7 (10) isolated from *A. putrefaciens*.

The ^1H NMR spectrum of MTK-7(c) is shown in Fig. 3. The signal of peak e (2.62 ppm) assigned as the proton of SCH_3 was similar to that of MTK-7(H_4) (2.64 ppm) isolated from *Hydrogenobacter thermophilus* TK-6 (9). This signal is unique to MTKs and is significantly different from that of MK-7(d) (2.17 ppm) (data not shown). Based on mass spectrometry analysis, all the isoprene units of MTK-7(c) described in this report were found to be unsaturated. Although MTK-7(H_4) is different from MTK-7 in that the former has two saturated isoprene units at the end of seven isoprene units, the structure of the naphthoquinone part is expected to be the same based on the

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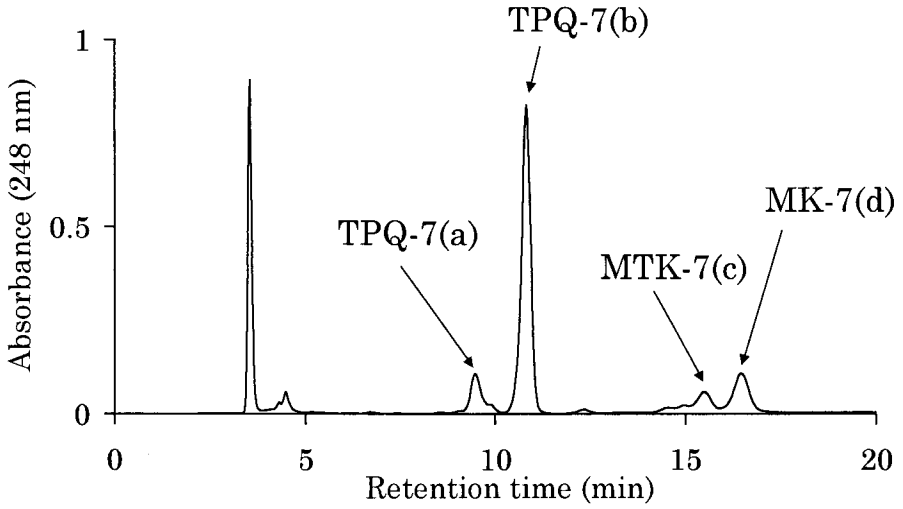

FIG. 1. HPLC chromatogram of a neutral lipid fraction from *T. acidophilum* HO-62. A Shiseido (Tokyo, Japan) Capcell pak silica UG80 column (4.6 by 250 mm) attached to an HPLC system (monitored at 248 nm) was used. The sample was separated using hexane-diethylether-trifluoroacetic acid (99:1:0.002, vol/vol/vol) at a flow rate of 0.86 ml · min^{-1}.

NMR data. MTK-7, which was tentatively assumed to be unsaturated, was found in the microbial mat which existed in the hot spring reported by Hiraishi et al. (8). However, there have been no reports on MTK-7 obtained from any isolated bacteria.

The samples used for the analysis of the quinone content under different growth conditions were extracted from cells of a 30-ml culture in the presence of sulfur (0.1 g · ml^{-1}) at 57°C. Aerobic culture was performed with an aeration flux of 55 ml · min^{-1}. Air was replaced by N_2 gas for anaerobic culture. The extracted crude lipid was directly applied onto the HPLC col-

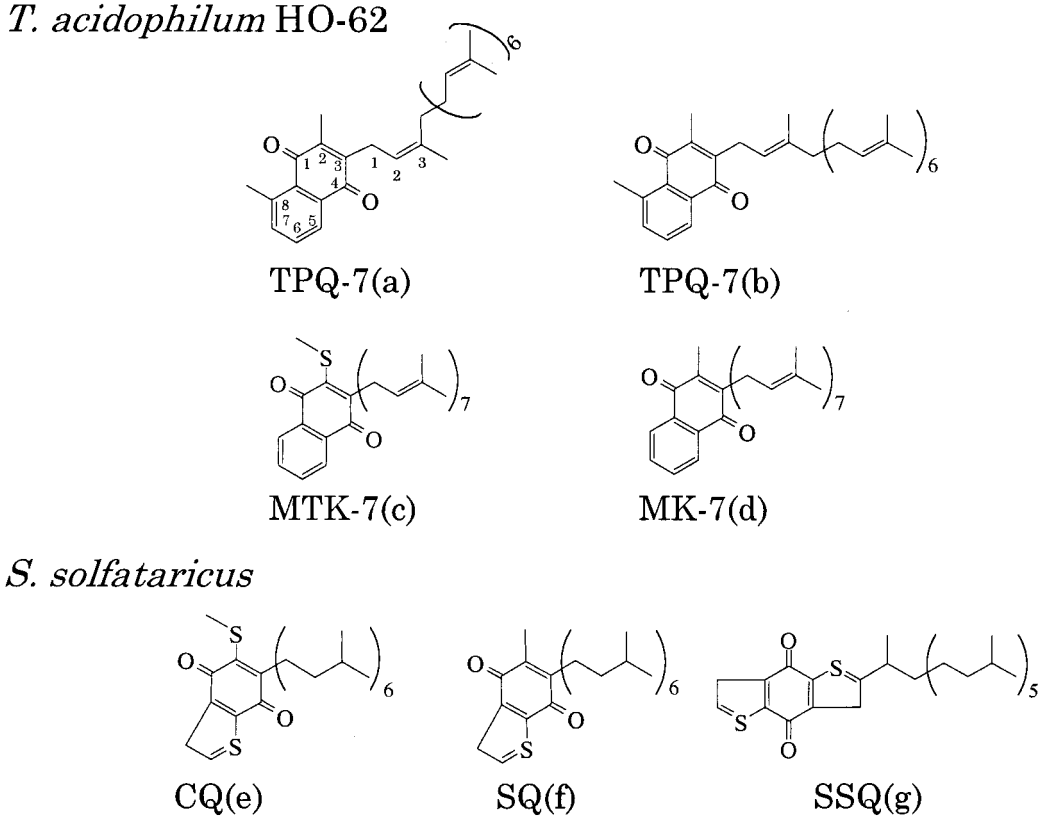

FIG. 2. Chemical structures of quinones elucidated from the results obtained in this report [TPQ-7(a) and -7(b) and MTK-7(c) and MK-7(d)] and the structures identified by Nicolaus et al. (13) [CQ(e), SQ(f), and SSQ(g), where SQ is sulfolobusquinone and SSQ is tricyclic quinone].

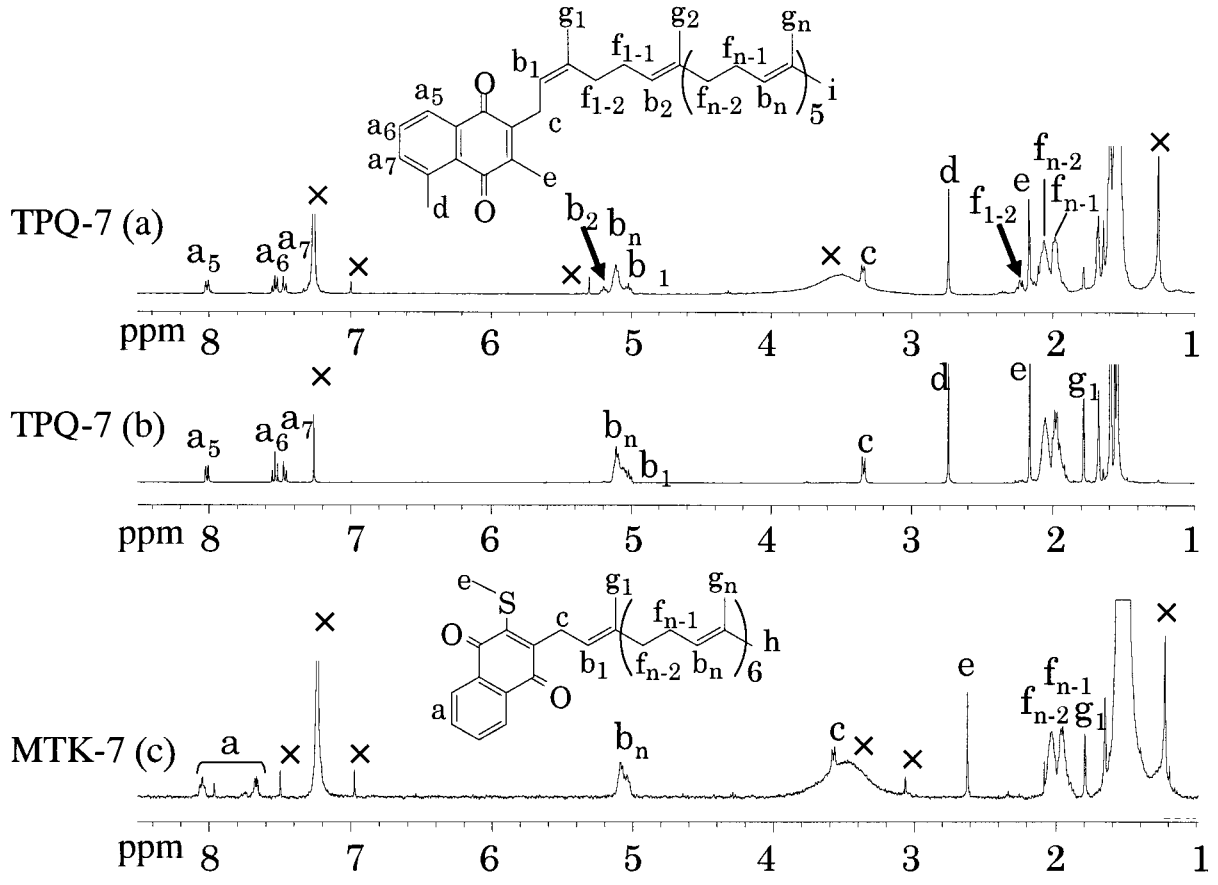

FIG. 3. ^{1}H NMR spectra (400 MHz). Shown are results of TPQ-7(a) in $CDCl_3$, 128 scans; TPQ-7(b) in $CDCl_3$, 16 scans; and MTK-7 (c) in $CDCl_3$, 128 scans. All spectra were recorded at an ambient temperature. Signals of impurity, water, or solvent are denoted by a ×. In the TPQ-7(a) spectrum, signals different from those of TPQ-7(b) are denoted by arrows.

umn. The relative amount of quinones was estimated from the peak area monitored at 248 nm. Freshly presented samples tend to show a higher amount of 2-*trans*-TPQ-7 [TPQ-7(b)] than of 2-*cis*-TPQ-7 [TPQ-7(a)]. Because 2-*cis*-TPQ-7 [TPQ-7(a)] seemed to be formed by isomerization of 2-*trans*-TPQ-7 [TPQ-7(b)] during the purification process, the percentage of TPQ-7 was expressed as the sum of the amount of *cis* and *trans* forms. Under aerobic conditions, the relative amounts of

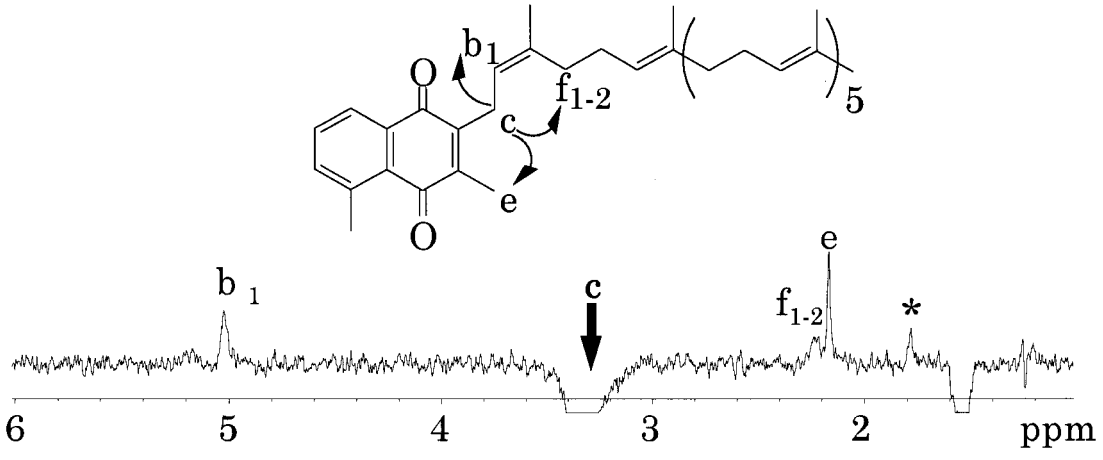

FIG. 4. 1D-NOE spectrum (400 MHz) of TPQ-7(a) presaturated at peak c (arrow) in $CDCl_3$. A total of 32,000 scans were accumulated at an ambient temperature. The peak denoted by an asterisk probably corresponds to TPQ-7(b) contaminating the TPQ-7(a) sample.

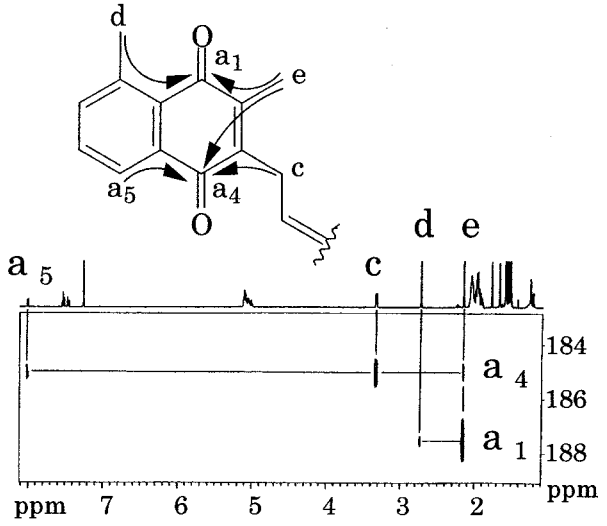

FIG. 5. Heteronuclear multiple-bond correlation spectrum (500 MHz) of TPQ-7(b). The horizontal and vertical axes show the ^{1}H chemical shift and ^{13}C chemical shift, respectively (only for carbonyl carbons). A total of 1,024 scans were accumulated at an ambient temperature.

TPQ-7, MTK-7, and MK-7 were 34, 34, and 33%, respectively (Fig. 6). While under anaerobic conditions, 97% of total quinone was TPQ-7 and only 3% was MK-7. TPQ-7 was also predominant in cells statically cultured. TPQ-7 was produced under anaerobic conditions. The relative amounts of MK-7 and MTK-7 were higher under aerobic conditions than those under anaerobic conditions.

In the case of *Escherichia coli*, ubiquinone, a derivative of benzoquinones, is used for aerobic respiration, because it has a high redox potential, while for anaerobic respiration, MK is used due to its low redox potential (7, 12). Although the redox potential of quinones in *Thermoplasma* was not estimated, Fig. 6 shows that different naphthoquinones are used depending on the oxygen supply in *Thermoplasma*.

The relative amounts of CQ (caldariellaquinone) and SQ (sulfolobusquinone) in *Solfolobus solfataricus* and *Desulfurolobus ambivalens* were influenced by the oxygen supply. The amount of CQ increased under aerobic conditions (13, 16). The structure of CQ is similar to that of MTK: they both have a methylthio group (SCH_3) attached to the second position in the quinone ring. The amount of MTK in *T. acidophilum* also increased under aerobic conditions. Accordingly, the methylthio group in the quinone ring appears to play an important role in growth under aerobic conditions.

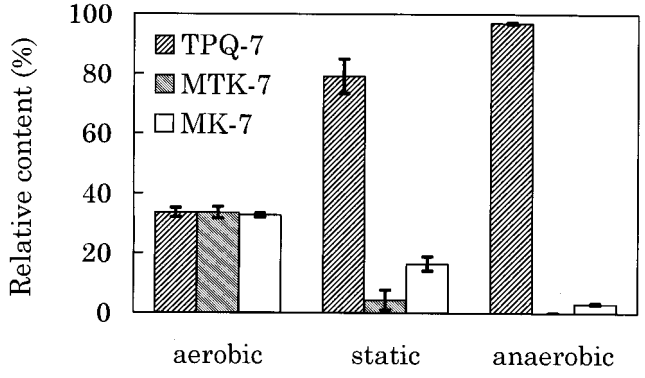

FIG. 6. Quinone composition in *T. acidophilum* cells cultured under different air supplies. Each value is a mean obtained from three independent experiments (error bars, standard deviations).

The amount of MK-7 in *T. acidophilum* HO-62 was also increased under aerobic conditions. The only difference between MK-7 and TPQ-7 is a CH_3 at position 8. The amount of MK-7 may be increased under aerobic conditions by suppressing methylation, which must occur at the last step of biosynthesis of TPQ-7. These results suggest that *T. acidophilum* HO-62 not only can grow in an anaerobic environment by producing an increased amount of TPQ-7 but also can adapt to an aerobic environment by producing MK-7 and MTK-7. It will be interesting to investigate the regulation of the biosynthesis process under different growth conditions.

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