JOURNAL OF BACTERIOLOGY, Nov. 1985, p. 911–913
0021-9193/85/110911-03$02.00/0

Vol. 164, No. 2

NOTES

Identity of the Quinone in *Bacillus alcalophilus*

R. MEGANATHAN* AND RICHARD COFFELL

Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115

Received 31 May 1985/Accepted 24 July 1985

Every *Bacillus* species so far examined contains menaquinone as the sole quinone. In contrast, the alkalophilic *Bacillus alcalophilus* has been reported to be unusual in containing ubiquinone rather than menaquinone. In this communication, we demonstrate that *B. alcalophilus*, like all the other bacilli, contains menaquinone as the only quinone.

The alkalophilic bacteria pose interesting questions of physiology (5, 7). These organisms grow best in the pH range of 9 to 12 but are able to maintain their internal pH at 9.5 or below (8). The bioenergetic issues raised by these organisms are of particular concern. For example, the well-studied organism *Bacillus alcalophilus* has been reported to contain extraordinarily high levels of cytochromes (9).

However, there have been conflicting reports concerning the quinone component(s) of *B. alcalophilus*. Hess et al. initially identified the quinone as menaquinone (4). Other investigators subsequently suggested that the growth conditions used by Hess et al. (4) would not have allowed growth of this species; furthermore, these workers claim to have identified ubiquinone as the only quinone present (7, 9).

Since every *Bacillus* species so far examined contains menaquinone as the sole quinone (1, 7), the reported presence of ubiquinone in *B. alcalophilus* (7, 9) is very unusual. We have therefore undertaken a reexamination of the quinone composition of this organism.

(A preliminary report of these findings has appeared [R. Meganathan and R. Coffell, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, K115, p. 190].)

B. alcalophilus ATCC 27647, NCTC 4553 strain Vedder, obtained from the American Type Culture Collection, was the same strain used by Hess et al. (4) and Lewis et al. (9). The organism failed to grow aerobically in the tryptic soy broth (Difco Laboratories, Detroit, Mich.) medium used by Hess et al. (4). However, when the medium was made alkaline by adjusting the pH to 10.5 with NaOH, good growth occurred. Thus, as pointed out by Krulwich and Guffanti (7), this organism could not have grown under the conditions used by Hess et al. (4). These workers presumably examined a contaminant.

For the isolation of the quinone, the organism was grown at 30°C in either the Tris-potassium phosphate medium at pH 10.5 as described by Guffanti et al. (3) or in nutrient broth adjusted to pH 10.5. The cells were centrifuged at 5,000 rpm for 10 min in an International centrifuge with an A-57 rotor. The pellet was resuspended in 0.25 volume of 0.02 M potassium phosphate buffer, pH 7.0, and centrifuged. The cells after resuspension in a small quantity of distilled water were lyophilized.

The quinone was extracted from the lyophilized cells by a modification of the procedure described by Collins et al. (2). Approximately 200 mg of lyophilized cells was mixed with 40 ml of chloroform-methanol (2:1, vol/vol), and the suspension was stirred continuously with a magnetic stirring bar overnight in complete darkness at room temperature. The suspension was filtered through Whatman no. 1 filter paper, and the filtrate was evaporated under reduced pressure. The residue was extracted with acetone and again dried under vacuum. The dried residue was dissolved in a small volume (about 500 μl) of ethyl acetate, and the solution was spotted

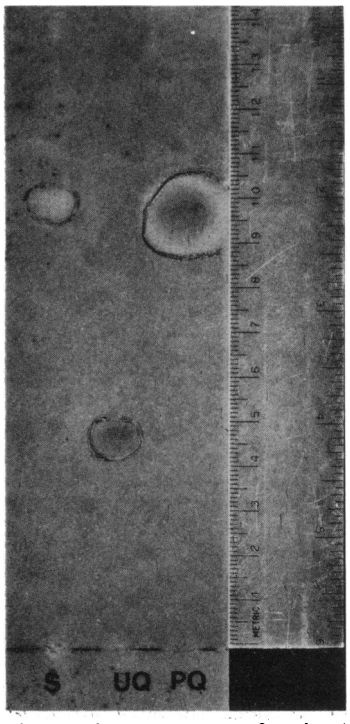

FIG. 1. Thin-layer chromatogram of quinones. Lanes: S, quinone isolated from *B. alcalophilus*; UQ, ubiquinone; PQ, phylloquinone.

* Corresponding author.

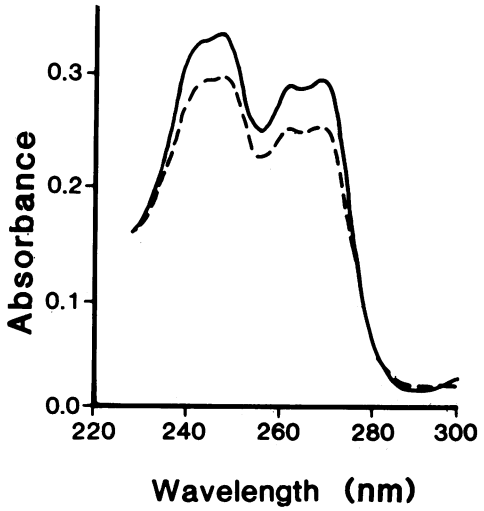

FIG. 2. UV absorption spectra of quinones. ——, Spectrum for phylloquinone; – – –, spectrum for quinone isolated from *B. alcalophilus*.

on 250-μm thick anasil OF thin-layer plates (Analabs, Northaven, Conn.). Ubiquinone-10 and phylloquinone were used as standards. The plates were developed in petroleum ether (boiling point, 36.9 to 54.5°C)–diethyl ether (85:15, vol/vol). The spots were visualized and photographed under UV light at 250 nm (Fig. 1). The phylloquinone standard and the quinone isolated from *B. alcalophilus* both had R_fs of 0.6, whereas the ubiquinone had an R_f of 0.3.

The quinone spot from *B. alcalophilus* was scraped from the plate and was eluted with chloroform; this solution was evaporated under reduced pressure. The dried residue was dissolved in absolute ethanol to be used for spectral studies (Fig. 2). Since the absorption of UV light is a function of the naphthoquinone ring only, phylloquinone was used as a standard. The solid line shows the spectrum for phylloquinone, and the dotted line shows the spectrum for the quinone from *B. alcalophilus*. The spectra were identical, thus confirming the results obtained with thin-layer chromatography.

Since it is known that under the conditions used for isolation the quinone will be fully oxidized and since the spectrum for the reduced quinone shows characteristic

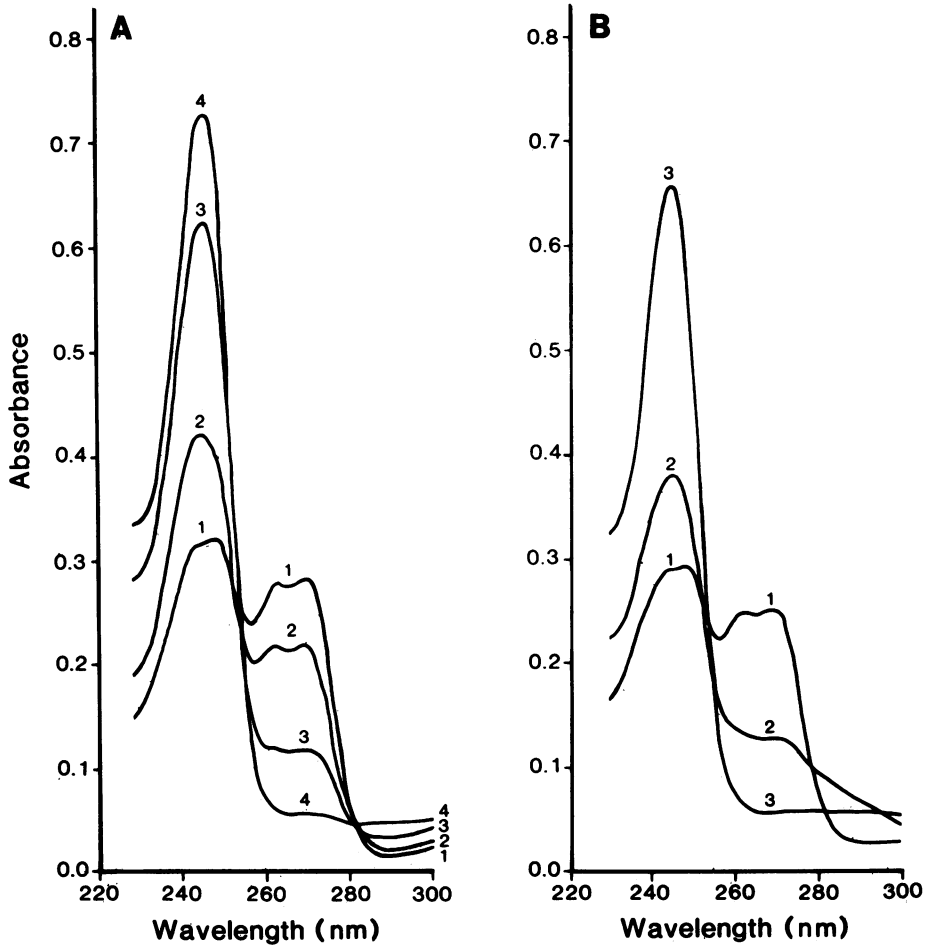

FIG. 3. Borohydride-reduced UV spectra of phylloquinone and the quinone from *B. alcalophilus*. To 1.5 ml of the quinone solution in absolute ethanol was added 2 μl of 0.4 M sodium acetate buffer, pH 5.4, to prevent autooxidation (6). (A) Spectra of phylloquinone. Lines 1 to 4 indicate the reduction achieved by the addition of 0, 2, 4, and 6 μl, respectively, of 5 mg of KBH_4 per ml (6). (B) Spectra of the quinone isolated from *B. alcalophilus*. Lines 1 to 3 indicate the reduction achieved by the addition of 0, 2, and 4 μl, respectively, of 5 mg of KBH_4 per ml (6).

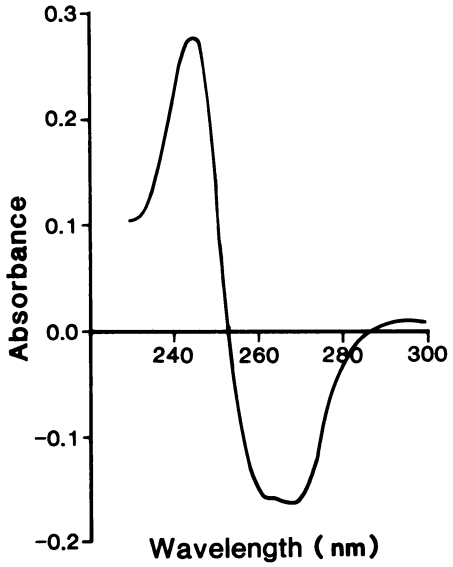

FIG. 4. Oxidized-versus-reduced UV spectrum of *B. alcalophilus* quinone. The oxidized solution of quinone was added to both cuvettes. The quinone solution in the experimental compartment was reduced with 4 μl of KBH_4 as described for Fig. 3.

changes, reduction with borohydride was carried out as described by Kröger and Dadák (6). The results with phylloquinone are shown in Fig. 3A. The spectrum of fully oxidized phylloquinone is superimposed on the reduced spectra obtained after the addition of progressively increasing amounts of potassium borohydride. The fully oxidized phylloquinone showed peaks at 245 and 265 nm. On reduction with increasing concentrations of borohydride, the 245-nm peak increased with a decrease in the 265-nm peak. At a concentration of 30 μg of borohydride, the 265-nm peak completely disappeared. The spectra obtained on borohydride reduction of the quinone isolated from *B. alcalophilus* are shown in Fig. 3B. It can be seen that the spectra are identical to those obtained with phylloquinone (Fig. 3A).

The thin-layer chromatographic and UV spectral properties of the quinone isolated from *B. alcalophilus* clearly showed that the quinone is indeed menaquinone. Since oxidized-versus-reduced spectra of menaquinones are quite characteristic, an oxidized-versus-reduced spectrum of the isolated quinone was recorded as a further check. The spectrum was identical to that obtained by Kröger and Dadák (6) for menaquinone isolated from *Bacillus megaterium* (Fig. 4).

Since it must be concluded that Hess et al. (4) were not working with *B. alcalophilus*, our work provides the first evidence for the presence of menaquinone and absence of benzoquinone in this bacterium. The earlier reports (7, 9) of the presence of ubiquinone must rest on a misidentification of the quinone, and *B. alcalophilus* cannot be regarded as exceptional with respect to its quinone component.

We thank Ronald Bentley for reviewing the manuscript.

LITERATURE CITED

1. **Collins, M. D., and D. Jones.** 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. Microbiol. Rev. **45**:316–354.
2. **Collins, M. D., T. Pirouz, M. Goodfellow, and D. E. Minnikin.** 1977. Distribution of menaquinones in actinomycetes and corynebacteria. J. Gen. Microbiol. **100**:221–230.
3. **Guffanti, A. A., P. Susman, R. Blanco, and T. A. Krulwich.** 1978. The protonmotive force and α-aminoisobutyric acid transport in an obligately alkalophilic bacterium. J. Biol. Chem. **253**:708–715.
4. **Hess, A., R. Holländer, and W. Mannheim.** 1979. Lipoquinones of some spore-forming rods, lactic-acid bacteria and actinomycetes. J. Gen. Microbiol. **115**:247–252.
5. **Horikoshi, H., and T. Akiba.** 1982. Alkalophilic microorganisms. A new microbial world. Springer-Verlag, New York.
6. **Kröger, A., and V. Dadák.** 1969. On the role of quinones in bacterial electron transport. The respiratory system of *Bacillus megaterium*. Eur. J. Biochem. **11**:328–340.
7. **Krulwich, T. A., and A. A. Guffanti.** 1983. Physiology of acidophilic and alkalophilic bacteria. Adv. Microb. Physiol. **24**:173–214.
8. **Krulwich, T. A., K. G. Mandel, R. F. Bornstein, and A. A. Guffanti.** 1979. A non-alkalophilic mutant of *Bacillus alcalophilus*. Biochem. Biophys. Res. Commun. **91**:58–62.
9. **Lewis, R. J., R. C. Prince, P. L. Dutton, D. B. Knaff, and T. A. Krulwich.** 1981. The respiratory chain of *Bacillus alcalophilus* and its nonalkalophilic mutant derivative. J. Biol. Chem. **256**:10543–10549.