Short Communication

GROWTH OF ESCHERICHIA COLI TM–1 ON NATURAL PHOSPHONIC ACIDS AND THEIR RELATED COMPOUNDS

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2-Aminoethylphosphonic acid (ciliatine) is well known as a C-P compound first isolated from biological materials (1, 2). The linkage of C-P in aminoalkylphosphonic acids is very stable, withstanding boiling with 6 N HCl, like the linkage of C-S in taurine. Limited investigations with rats (3–7) and with mouse and carrot preparation (8) suggest that higher animals and plants are unable to break this C-P bond.

Our previous papers reported the isolation from gastrointestinal microorganisms of sheep (9, 10) of Escherichia coli Tm–1 and Tm–2 having the ability to utilize the phosphorus of ciliatine for growth. The purpose of this investigation was to compare the growth of Escherichia coli Tm–1 on natural C-P compounds and their related compounds.

Ciliatine was synthesized by the method of Kosolapoff (11) (a part used here was a gift from Dr. H. Kameyama of Kumamoto Woman’s University). Phosphonooalanine (AEnP), aminomethylphosphonic acid (AMP), N-monomethylciliatine (MM-ciliatine), N-dimethylciliatine (DM-ciliatine), N-trimethylciliatine (TM-ciliatine), and 1-aminoethylphosphonic acid (1-AEPn) were gifts from Dr. A. Isbell of the Department of Chemistry, Texas A & M University. The following synthetic medium was used for growth: 7.8 g Na lactate, 4 g glycerol, 5 g NaCl, 2 g KCl, 1 g NH₄Cl, 0.09 g MgCl₂, 0.1 g CaCl₂, 0.01 g gelatin, 10 mg MgSO₄·7H₂O, and distilled water to bring the total volume to 1 liter, pH adjusted to 7.0. Stocks of the bacteria were maintained on the synthetic medium-agar slants. Stationary phase cells grown on the medium were inoculated to a density of 1.4 × 10⁴ cells into the fresh medium containing various quantities of organophosphonic acids.

Bacteria were cultured for 20 hr on a shaker (Type FFO, Hitachi Ltd., Tokyo)
at 270 rotations/min at 37°. The growth of the bacteria was measured by spectrophotometry at 420 nm with a Shimadzu spectrophotometer using a cuvette with a light path of 1 cm. After 20 hr of incubation, the cells were collected by membrane (Millipore, 0.22 μm) filtration. Ciliatine remaining in the culture filtrate was determined as described in our previous paper (3). Other phosphonates remaining in the culture filtrate were determined by the difference between the total phosphorus and the acid-labile phosphorus according to the method of ALAM and BISHOP (12). The uptake of phosphonate was calculated from the amount of phosphonate remaining in the culture filtrate.

The effect of the concentration of phosphonates and orthophosphate as a sole source of phosphorus on the growth of E. coli Tm–1 is shown in Fig. 1. A maximal growth of the bacteria was obtained in the medium containing orthophosphate.

![Graph showing maximal growth of Escherichia coli Tm–1 on various concentrations of natural phosphonic acids and their related compounds.](image)

**Fig. 1.** Maximal growth of *Escherichia coli* Tm–1 on various concentrations of natural phosphonic acids and their related compounds.

Stationary phase cells grown on synthetic medium were inoculated to a density of $1.4 \times 10^4$ cells into a synthetic medium containing various quantities of phosphonates. Bacteria were cultured at 37° for 20 hr on a rotary shaker. The growth of the bacteria was measured spectrophotometrically at 420 nm. Composition of the medium used here is given in the text. Symbols: 1, orthophosphate; 2, ciliatine; 3, MM-ciliatine; 4, DM-ciliatine; 5, AMP; 6, TM-ciliatine; 7, AEnP; 8, 1-AEPn.

All phosphonates, except 1-AEPn, supported bacterial growth; the growth was the highest on ciliatine, followed by MM-ciliatine, DM-ciliatine, AMP, TM-ciliatine, and AEnP in the order of decreasing ability to support the growth.

A maximal growth on ciliatine was observed in media ranging in concentra-
tion from 1.2 to 2 mM, while HARKNESS (13) and ALAM and BISHOP (12) reported a lower concentration (0.2–0.4 mM) of ciliatine required for an optimum growth of *E. coli* Crooke's (ATCC 8739) which reduces dehydroascorbic acid to ascorbic acid.

The failure to observe bacterial growth on 1-AEPn would be explained by the inhibitory effect of this phosphonate on the cell wall synthesis of *Proteus vulgaris* observed by DULANEY (14).

Maximal growth on other organophosphonic acids was obtained with MM-ciliatine at 2 mM, DM-ciliatine at 3 mM, AMP at 2 mM, and TM-ciliatine at 3–4 mM. The phosphonic acids used here occur in nature with the exception of AMP and 1-AEPn. The phosphonate uptake by *E. coli* Tm–1 grown on the synthetic medium containing various quantities of phosphonates is shown in Fig. 2. The uptake of phosphonates into the cell body was high with ciliatine and orthophosphate, but lower in the case of AEEnP and TM-ciliatine. 1-Aminoethylphosphonic acid was scarcely taken up by *E. coli* Tm–1.

![Fig. 2. Phosphonate uptake by *Escherichia coli* Tm–1 grown on synthetic medium containing various quantities of phosphonates.](image)

Stationary phase cells grown on a synthetic medium were inoculated to a density of $1.4 \times 10^4$ cells in a synthetic medium containing various quantities of phosphonates. Bacteria were cultured at 37° for 20 hr on a rotary shaker. Phosphonates remaining in the culture filtrate were measured by the method described by ALAM and BISHOP (12). The symbols used here are the same as those in the Fig. 1.

From the above results obtained here, it was found that *E. coli* Tm–1 is able to well utilize phosphorus of natural C-P compounds, but 1-aminoethylphosphonic acid was scarcely utilized. The mineralization of phosphonates by microorganisms may well contribute to the circulation of phosphorus in nature, because higher animals and plants seem to be unable to break the C-P bond.
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REFERENCES