Defined Liquid Minimal Medium for *Caryophanon latum*

ROGER A. KELE and ELIZABETH MCCOY

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 19 April 1971

A defined medium has been developed for the trichome-forming bacterium, *Caryophanon latum*. Acetate is the main carbon and energy source, and either N-glutamate, or N-NH₄ can be used as the nitrogen source.

In 1939, Peshkoff described a new bacterium, *Caryophanon latum* Peshkoff, obtained from fresh cow dung collected near Moscow, Russia (1). The distinguishing features of *C. latum* are its large size (3 by 10 to 30 μm) and its normal growth habit in the form of trichomes. Much work has been devoted to the cytology of this organism (e.g. 2, 3) but relatively little to its physiology. In 1962, Provost and Doetsch (4) developed the first completely liquid medium which, with sufficient aeration, supported good growth of eight out of nine of their cultures. However, growth in this medium results in deformation of the cells by deposition of poly-β-hydroxybutyrate (PHB) and early lysis of the cells. Therefore, variations of the medium were sought in the hope of improving the morphology. This was achieved with a semisynthetic organic medium with the following composition per liter: Edamin (enzymatic digest of lactalbumin, Sheffield), 10.0 g; sodium acetate, 5.0 g; K₂HPO₄, 1.0 g; MgSO₄·7H₂O, 0.27 g; thiamine·HCl, 0.2 mg; and biotin, 0.05 mg. The medium was prepared in 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8.0. Testing butyrate as a growth stimulant as previously suggested (4), we found that with acetate in the medium growth was independent of the amount of butyrate added, but, in the presence of butyrate, growth was proportional to the amount of acetate added. Thus butyrate is not essential whereas acetate is.

By substituting amino acids and minerals for Edamin, a completely defined medium was devised which has the following composition per liter: sodium acetate, 5.0 g; monosodium glutamate, 1.0 g; NH₄Cl, 1.0 g; K₂HPO₄, 1.0 g; Na₂S·9 H₂O, 0.01 g; thiamine·HCl, 0.2 mg; biotin, 0.05 mg; and mineral salts—nitritoltriacetic acid, 0.15 g; MgSO₄, 0.3 g; CaCl₂, 0.01 g; and FeSO₄, 0.01 g (anhydrous salts). The medium is prepared in double-distilled water buffered at pH 7.8 with 0.01 M Tris. The vitamin and mineral solutions are added aseptically from 100× and 10× stock solutions after autoclaving. Cultures were grown at 25 C in 6 ml of medium in slanted test tubes on a rotary shaker at 250 rev/min (4). Growth was measured photometrically at 660 nm.

The amount of growth in the defined medium was proportional to the amount of acetate present, up to 2% (Fig. 1). No growth occurred with glutamate at 0.1% in the absence of acetate (curve A), yet glutamate itself is not essential for growth, since N-NH₄ and C-acetate are sufficient (Fig. 2; 0% glutamate curve). Growth was enhanced, however, with 0.25 and 0.5% glutamate, suggesting that *C. latum* can utilize some C-glutamate in the presence of acetate. It is reasonable to conclude that acetate is essential as a carbon or energy source (or both) for *C. latum*.

Glutamate at 0.1% supported fair growth in the absence of NH₄Cl indicating that it can be utilized as the sole nitrogen source. However, growth was stimulated considerably by as little as 0.01% NH₄Cl and more so by 0.05 and 0.1%. The NH₄Cl probably relieved a nitrogen deficiency at the 0.1% glutamate level (0.0083% N in the medium).

The defined medium was developed by using the R4 strain of *C. latum*. To determine its general suitability, six other strains were tested. Four of them (1671, G139, G380, and E1) were our own isolates, obtained by the method of Pringsheim and Robinow (3); a fifth strain was ATCC culture 15219, from D. Dean, Baldwin-Wallace College, Berea, Ohio; and the sixth strain (LW) was received from Lois Weber, Pennsylvania State University. Five out of seven strains grew vigorously in the medium, and could be repeatedly subcultured via loop transfer. The ATCC 15219 and
strain E1 failed to grow in the medium, although three and four separate attempts were made. Whether they have additional growth requirements or only different quantitative requirements is not known. The morphology of the strains which grew well was somewhat abnormal at first, the trichomes being long, crooked, and somewhat thin, but they became actively motile and normal after several subcultures. There was no problem of excessive PHB deposit.

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison.

**LITERATURE CITED**

1. Peshkov, M. A. 1939. Cytology, karyology and cycle of development of new microbes Caryophanon latum and Caryophanon tenue. Mihl Compt. Rend. (Doklady) Acad. Sci. U.S.S.R. 25:244–247.

2. Peshkov, M. A., L. A. Levchenko, and A. S. Sharkova. 1966. Mode of formation and ultrafine structure of heteromorphic forms of Caryophanon latum Peshkoff. Microbiology 35:888–892.

3. Pringsheim, E. G., and C. F. Robinow. 1947. Observations on two very large bacteria, Caryophanon latum Peshkoff and Lineola longa (nomem provisorium [sic]). J. Gen. Microbiol. 1:267–278.

4. Provost, P. J., and R. N. Doetsch. 1962. An appraisal of Caryophanon latum. J. Gen. Microbiol. 28:547–557.