

Some Growth Factors for *Fusobacterium polymorphum*

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Fusobacterium polymorphum Knorr (ATCC 10953) is an anaerobic, spindle-shaped organism found in the oral cavity of normal humans or in lesions of Vincent's infection (*Bergey's Manual*, 7th ed., p. 438). The fusobacteria are characterized by fastidious growth requirements which have not been completely defined. Omata (2) demonstrated that strains of *F. nucleatum* need adenine or xanthine, pantothenic acid, and tryptophan for growth. We have found that *F. polymorphum* also requires tryptophan, a purine (adenine or xanthine), and pantothenic acid. Although its nutritional demands are similar, *F. polymorphum* does not grow in Omata's medium. However, if glucose is heat-sterilized with the medium, if the adenine concentration is reduced, and if sodium bicarbonate is added to Omata's medium after sterilization, *F. polymorphum* will initiate growth in this medium. Further determination of the nutritional requirements of *F. polymorphum* was made by selective omission of single components from a semisynthetic medium.

Cultures of *F. polymorphum* were maintained in Brewer's modified fluid thioglycolate medium (Fisher). Inocula were washed three times with cold sterile saline and suspended to a concentration of 10^8 to 2×10^8 cells per ml. Media, prepared in 10-ml amounts in culture tubes (18 by 150 mm), were inoculated with 0.1 ml of the cell suspension, mixed, and incubated in an atmosphere of 5% CO_2 and 95% N_2 at 37 C.

The growth of *F. polymorphum* in modified Omata medium is shown in Fig. 1. The maximum rate of growth and turbidity were achieved when glucose was autoclaved in the medium, when 12.5 μmoles (per ml) of sodium bicarbonate was added after sterilization, and when the adenine concentration was reduced to 0.5 μmole per ml. Higher concentrations of adenine, needed for maximum growth of *F. nucleatum*, were inhibitory. Growth was not initiated when filter- or heat-sterilized glucose was added to previously sterilized basal medium.

In the absence of bicarbonate, the lag was

longer and growth was less than that obtained in its presence. Various species of *Propionibacterium* also require, for initiation of growth, either substances produced by reaction of glucose with other medium components during heating or exogenous sodium bicarbonate (1; R. S. Coles and H. C. Lichstein, unpublished data).

Selective omission of components of the semi-synthetic medium A (Table 1) indicated that

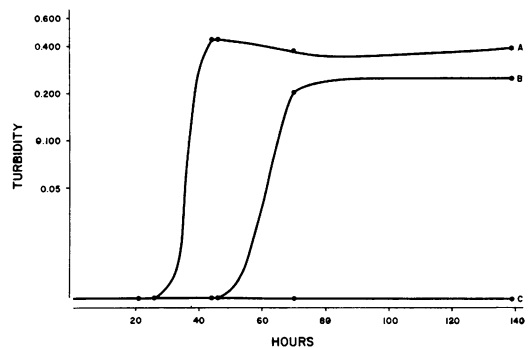


FIG. 1. Growth of *F. polymorphum* in modified Omata's medium. Turbidity was measured with a Coleman (model 9) nephocolorimeter at 655 nm. (A) Growth in the presence of bicarbonate, 0.5 μmole of adenine per ml, glucose autoclaved in medium. (B) Growth in the absence of bicarbonate, with 0.5 μmole of adenine per ml, and with glucose autoclaved in medium. (C) Growth in medium containing glucose added aseptically after autoclaving medium with or without adenine or bicarbonate or both.

Thiotone, the amino acids cystine, serine, lysine, and glutamic acid, together with acetate, phosphate, bicarbonate, and glucose, which need not be autoclaved in the medium, are essential for growth (Table 2). Thiotone cannot be replaced by single amino acids, purines, pyrimidines, or vitamins, or by mixtures of these substances. However, it supplies the organism with pantothenic acid, adenine or xanthine, and tryptophan. Because it does not supply adequate quantities of

TABLE 1. *Composition of medium A for demonstration of amino acid requirements of F. polymorphum*

Component	Amt per liter
Thiotone (BBL).....	1 g
K ₂ HPO ₄	1 g
KH ₂ PO ₄	1 g
Sodium acetate.....	9.5 g
L-Cystine.....	0.2 g
L-Serine.....	0.4 g
L-Lysine.....	1.15 g
L-Glutamic acid.....	3.08 g
Sodium bicarbonate ^a	12.5 mmoles
Glucose ^a	12.5 mmoles

^a Added aseptically after autoclaving of medium.

cystine, serine, lysine, and glutamic acid, suboptimal levels of Thiotone can be used in the semi-synthetic medium for studying nutritional requirements of *F. polymorphum*. On increasing the concentration of Thiotone, not only is the total growth increased but the medium no longer requires supplementation with amino acids.

These studies indicate that *F. polymorphum* requires serine, lysine, and glutamic acid in addition to the amino acids found essential for *F. nucleatum* by Omata. In addition, sodium bicarbonate and, under certain conditions, products of reaction under heat between glucose and other medium components are needed.

TABLE 2. *Growth of F. polymorphum in medium A*

Deletion from medium	Turbidity ^a
None.....	0.191
Thiotone.....	0.013
Phosphate.....	0
Sodium acetate.....	0.090
L-Cystine.....	0
L-Serine.....	0
L-Lysine.....	0.087
L-Glutamic acid.....	0.064
Sodium bicarbonate ^b	0
Glucose ^b	0.013

^a Growth after 48 hr of incubation (determined at 655 nm in a Coleman model 9 nephcolorimeter). Growth did not continue beyond the turbidities recorded for another 96 hr of incubation.

^b Added after sterilization of other medium components.

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2. Omata, R. R. 1959. Studies on the nutritional requirements of the fusobacteria. II. Requirements for pantothenate and purines. *J. Bacteriol.* 77:35-37.