Note

Isolation of a Formate-assimilating Bacterium and Its Vitamin B₁₂ FormationMasahiro OHSUGI, Michiko YAMADA,
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Since Rickes's discovery that *Actinomycetes* were able to produce extracellular vitamin B₁₂,¹⁾ the production of vitamin B₁₂ has been performed by fermentation of carbohydrates using *Actinomycetes* such as *Streptomyces olivaceus*,^{2,3)} bacteria such as *Bacillus megatherium*,⁴⁾ *Propionibacterium sharmanii*,^{5,6)} and *Pseudomonas denitrificans*,⁷⁾ and others such as red algae⁸⁾ and *Chlorella pyrenoidosa*.⁹⁾ Recently, many papers have appeared on the production of vitamin B₁₂ by fermentation of non-carbohydrates with hydrocarbon-utilizing bacteria,^{10~12)} propanediol-utilizing *Propionibacterium*,¹³⁾ propionate-acetate-utilizing *Rhodopseudomonas*¹⁴⁾ and methanol-utilizing bacteria.^{15~21)} Vitamin B₁₂ catalyzes the transfer of hydrogen or methyl groups as adenylyl or methyl co-bamide coenzyme forms, the former are known to participate in about 11 types of important biochemical reaction, and the latter in methionine, methane and acetic acid synthesis as reviewed.^{22~26)} However, little is known about the relationship between metabolism of C₁-compounds and vitamin B₁₂. Formate is an electron donor of methanogenic bacteria.²⁷⁾ Little is known about the metabolism of formate and the formation of vitamin B₁₂ in formate-assimilating bacteria. This communication deals with the formation of vitamin B₁₂ and the metabolite of formate by a formate-assimilating bacterium isolated from soil.

The medium contained formate 0.4%, (NH₄)₂SO₄ 0.2%, K₂HPO₄ 0.1%, KH₂PO₄ 0.1%, MgSO₄·7H₂O 0.05%, CoCl₂·6H₂O 6ppm and yeast extract 0.05%. The initial pH of the medium was adjusted to 7.0. To a test tube containing 4ml of the medium was added one loopful of soil. Test tubes were incubated for three to seven days at 28°C with continuous shaking (230 rpm). About one hundred strains were isolated from six hundred soil samples by repeating the enrichment culture technique. Vitamin B₁₂ in the culture fluid was measured by a biological assay with *Escherichia coli* Davis 113-3.^{28,29)}

Among isolated strains, twenty-three strains accumulated vitamin B₁₂ extracellularly in the range of 2.1~16.0ng per ml of medium. Of those strains isolated, strain 15-b which formed vitamin B₁₂ with a relatively good yield was selected for the present study. Strain 15-b could hardly utilize formate as a sole carbon source. Addition of organic nutrients such as malt extract, meat extract, yeast extract, casamino acids or C. S. L. at a concentration of 0.05% to the basal medium caused stimulation of growth. Among those nutrients tested, yeast extract was the most effective on growth and extracellular vitamin B₁₂ formation. As shown in Table I, growth and vitamin B₁₂ accumulation of this strain exclusively depend on the concentration of yeast extract added to medium. When formate was omitted from the medium throughout cultivation, no formation of vitamin B₁₂ was observed. Addition of Co⁺⁺ caused good formation of vitamin B₁₂. For example, after 119-hr cultivation at 0.1% of yeast extract, vitamin B₁₂ accumulation with and without ad-

TABLE I. EFFECT OF YEAST EXTRACT
CONCENTRATION ON GROWTH AND
VITAMIN B₁₂ ACCUMULATION

Concentration of yeast extract (%)	Cultivation time (hr)	Growth (OD at 470 nm)	Vitamin B ₁₂ (ng/ml)
0	66	0.052	0
0	114	0.071	0
0.05	66	0.128	3
0.05	114	0.202	7
0.1	66	0.299	13
0.1	114	0.351	15
0.3	66	0.680	20
0.3	114	0.770	24
0.5	66	0.930	46
0.5	114	1.070	70

TABLE II. TIME COURSE OF FORMATE
UTILIZATION AND ACETATE FORMATION

The medium was the same as described in the text except that concentrations of 0.2% formate and 0.2% yeast extract were used. Formate was determined according to the Lang method.³¹⁾ Acetate determination was performed with acetokinase.³²⁾

Cultivation time (hr)	Growth (OD at 470 nm)	Formate remaining (mg/ml)	Acetate formed (mg/ml)
0	0	1.60	0
26	0.78	1.05	0.38
47	0.80	0.93	0.47
92	0.97	0.87	0.66
139	0.92	0.82	0.15

dition of Co^{++} was 16.0 and 2.8 ng per ml, respectively. Several kinds of vitamins (1 $\mu\text{g}/\text{ml}$ added) and amino acids (100 $\mu\text{g}/\text{ml}$ added) were examined for their effects on the vitamin B_{12} formation, replacing yeast extract in the medium. The following compounds stimulated the formation of vitamin B_{12} to some extent (2.3~4.8 ng/ml formed): vitamin B_1 , biotin, L-aspartic acid, L-histidine, L-serine and L-valine. Of 24 kinds of fatty acids tested, only succinic acid appeared to stimulate vitamin B_{12} formation. δ -Aminolevulinic acid, mesaconic acid, 5,6-dimethylbenzimidazole, betaine and isopropanolamine, which are known to stimulate vitamin B_{12} formation, had no effect. The result of paper chromatography of vitamin B_{12} accumulated was identical with that of authentic cobalamin.³⁰⁾ The products formed from formate during cultivation were also examined by gas-liquid chromatography (Shimadzu GC-4BP (FID) apparatus). Only one peak was separated on a DEGS 10% column, with a retention time of 4.3 min. This peak was found to be identical with authentic acetic acid. The rates of formate utilization and acetate formation during cultivation were determined. Table II shows that during the initial stage of cultivation acetate was formed in an approximately equivalent amount in weight to formate utilized. After 92-hr incubation the level of acetate decreased probably because of the subsequent utilization of acetate. A cell-free extract of formate-grown cells of this strain possessed the activities of formaldehyde dehydrogenase and formate dehydrogenase. Therefore, it is assumed that the organism utilized formate to form formaldehyde. The mechanism of formation of acetate from formate and the taxonomical characteristics of strain 15-b are under investigation.

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