Characterization of Two New Facultative Methanotrophs

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Two new facultative methane-oxidizing bacteria have been isolated from lake water enrichments. The organisms have been characterized in terms of colony types, growth characteristics, the guanine plus cytosine content of their deoxyribonucleic acid, thin sections, oxidation rates, and carbon assimilation pathways. Methane-grown cells of both organisms contained intracytoplasmic membranes similar to those described as type II in other methanotrophic bacteria. Neither organism had such membranes when grown heterotrophically. Both organisms assimilated methane by way of the isocitrate lyase-negative serine pathway for formaldehyde incorporation. The enzymes of this pathway were high in specific activity in cells grown on methane and were at low levels in cells grown either on heterotrophic substrates or on heterotrophic substrates plus methane. It is proposed that both organisms be classified in the genus *Methylobacterium* as two new species, *Methylobacterium ethanolicum* and *Methylobacterium hypolimneticum*.

Methane-utilizing bacteria have been known to exist for over 70 years (25). However, until recently all isolates were obligate C-1 utilizers; that is, unable to utilize compounds containing carbon-carbon bonds as sole sources of carbon and energy (6, 27, 28).

Since 1974, two facultative methanotrophs have been reported, both of which contained peripheral (type II) internal membrane systems and utilized the serine pathway for formaldehyde fixation (20, 22). The isolation of these organisms has opened the way for detailed study of C-1 assimilation processes in methanotrophs (17-19). However, neither facultative methanotroph has proven suitable for genetic studies of methane oxidation, nitrogen fixation, and related functions.

This study was undertaken to isolate and characterize new strains of facultative methanotrophs which might prove more amenable to genetic analysis. Two such strains have been isolated and described in terms of their characteristics and taxonomic position.

MATERIALS AND METHODS

Media and growth conditions. The modified mineral salts medium (NMS) of Patt et al. (22) was used for most experiments. In some cases, a nitrogen-free mineral salts medium (NFMS) was used, which consisted of NMS excluding KNO_3 and substituting K_2HPO_4 (0.28 g/liter) for Na_2HPO_4 . Cells for deoxyribonucleic acid (DNA) isolation were grown on nutrient broth.

Succinate, acetate, glucose, xylose, galactose, sucrose, lactate, malate, and fructose were sterilized separately and added directly to the growth medium at 0.1% (wt/vol). Methanol (96%, vol/vol), ethanol (95%,

vol/vol), and propanol (100%, vol/vol) were filter sterilized and added directly to the growth medium at 0.5% (for methanol) or 0.2% (for ethanol and propanol).

A vitamin supplement (in $\mu\text{g/liter}$: biotin, 20; folic acid, 20; thiamine hydrochloride, 50; D-calcium pantothenate, 50; B_{12} , 1; riboflavin, 50; nicotinamide, 50) was routinely included in the medium for isolate H4-14. Although vitamins were not required by this isolate, they stimulated growth, especially after transfer from one substrate to another.

Yeast extract (0.005%, wt/vol) was routinely included in all media for the growth of isolate 37W.

Liquid cultures were incubated at 30°C on rotary shakers. Methane-grown cultures were initially gassed with a methane-air mixture of 90%-10%. As turbidity increased, the percentage of air was gradually increased to 50%. Cultures were gassed daily. Cultures were maintained on NMS plates, incubated under methane-air (90%-10%), and transferred weekly.

Isolation. Samples were taken aseptically throughout the water columns by using Cobet samplers (12). Portions (10 ml) were placed in flasks containing 30 ml of NFMS medium, gassed with methane-air mixtures (90%-10%), and incubated at 30°C with shaking for 2 to 3 weeks until turbid. Samples were streaked onto Millipore membrane filters soaked in NFMS medium and incubated under a methane-air atmosphere as before. In some cases a small amount of yeast extract (0.005%) was included in the medium. Colonies were alternately placed into liquid medium and streaked onto filters until pure cultures which grew well under both conditions were obtained.

Preparation of crude extracts. Frozen cells were thawed and suspended in 4 volumes of potassium phosphate buffer (20 mM, pH 6.8). Disruption was effected by ultrasonification (MSE, model MT20) using six 30-s bursts. The sonified extract was centrifuged at $20,000 \times g$ for 20 min, and the supernatant was used for enzyme assays.

Protein was estimated by the procedure of Lowry et al. (14).

Enzyme assays. The following enzymes were assayed by published methods: hydroxypyruvate reductase (D-glycerate:oxidized nicotinamide adenine dinucleotide oxidoreductase, EC 1.1.1.29) (13); serine-glyoxylate aminotransferase (L-serine:glyoxylate aminotransferase, EC 2.6.1.45) (1); glycerate kinase (adenosine triphosphate:D-glycerate 3-phosphotransferase, EC 2.7.1.31) (10); phosphoenolpyruvate carboxylase (orthophosphate:oxaloacetate carboxy-lyase [phosphorylating], EC 4.1.1.31) (2); serine transhydroxymethylase (5,10-methylene-tetrahydrofolate:glycine hydroxymethyl transferase, EC 2.1.2.1) (10, 17); methanol dehydrogenase (18); malyal coenzyme A (CoA) lyase (24); secondary alcohol dehydrogenase (11); isocitrate lyase (7); α -ketoglutarate dehydrogenase (2-oxoglutarate:lipoate oxidoreductase [decarboxylating and acceptor-succinylating], EC 1.2.4.2) (9); and hexulose phosphate synthase (4).

Nitrogen fixation. Nitrogen fixation was estimated in whole cells by using the acetylene reduction technique described by Dalton and Whittenbury (5). A 1-ml sample of cells grown on NFMS was incubated at room temperature with 0.5% (vol/vol) methanol under an atmosphere of 10% air and 90% helium in a 15-ml total volume. Acetylene (0.2 ml) was injected, and 1-ml samples were withdrawn at 5-min intervals. Ethylene in the samples was estimated using a Hewlett Packard Gas Liquid Chromatograph (model 5700 A), equipped with a 6-foot (ca. 1.83-m) Porapak R column, at 50°C.

Oxidation studies. A 20-ml volume of culture was washed in phosphate buffer (20 mM, pH 6.8) and resuspended in 2 ml of the same buffer. A 0.1-ml portion of the cell suspension was added to 0.8 ml of air-saturated buffer in an oxygen electrode (Rank Bros., Bottisham, England) and incubated at 37°C until endogenous respiration leveled off (5 to 10 min). Substrate (0.1 ml; 2%, wt/vol) was then added (final concentration, 0.2%, wt/vol), and the ensuing oxidation rate was followed for 4 to 5 min. Methane, ethylene, and propylene were added in the form of 0.1 ml of gas-saturated buffer.

DNA isolation. Frozen cells were suspended in TE buffer [tris(hydroxymethyl)aminomethane, 0.05 M; ethylenediaminetetraacetic acid, 0.02 M; pH 8.0] (5 g of cells per 90 ml of buffer) and lysed by incubation with pronase (10 μ g/ml) plus proteinase K (50 μ g/ml) plus sodium dodecyl sulfate (4%, wt/vol) for 60 min at 60°C. Pronase and proteinase K were previously heat treated at 80°C for 15 min before use. DNA was extracted twice with 0.5 volume of TE-saturated phenol and once with chloroform-isoamyl alcohol (24:1). Ribonuclease (heat treated at 80°C for 10 min) was added to a final concentration of 50 μ g/ml, and incubation was carried out for 10 min at 65°C. DNA was precipitated with 2 volumes of cold ethanol (95%) and suspended in 0.1 $\times$ SSC (standard saline citrate buffer, 0.15 M NaCl-0.015 M sodium citrate).

DNA was further purified by using a Sepharose column as described by Gibson and Ogden (8). Moles percent guanosine plus cytosine (G+C) was determined by using the T_m , as described by Marmur and Doty (15).

Materials. Pronase was obtained from Calbiochem (La Jolla, Calif.), and proteinase K came from Beckman (Palo Alto, Calif.). Malyal CoA was a generous gift from J. R. Quayle (Sheffield, England). All other chemicals were obtained from Sigma Chemical Co., St. Louis, Mo.

Electron microscopy. For thin sections, preparations were fixed in cold 3% glutaraldehyde (Taab Co., Reading, England) in 0.1 M cacodylate buffer, post-fixed in cold 1% OsO₄ in 0.1% M cacodylate buffer, dehydrated in gradients of alcohol, and embedded in Epon, using standard techniques.

RESULTS

Isolation. Two lakes in the Seattle area were used as sources of water for enrichment cultures. Hall Lake is a shallow (maximum depth, 15 m) meromictic lake containing an anaerobic hypolimnion. The thermocline at the time of sampling (December, 1978) was at 12 m. Lake Washington is a large, mesotrophic, holomictic lake containing an aerobic hypolimnion. The thermocline at the time of sampling (October, 1978) was at 20 m, with a maximum depth at the sampling point of 57 m. Enrichment cultures of samples from these two lakes produced three isolates. Of the three isolates obtained in this way, two were found to be facultative and were used for further studies.

One isolate, H4-14, came from an enrichment of a 12-m sample from Hall Lake. The other, 37W, was from an enrichment of a 37-m sample from Lake Washington. Although enrichments from lower depths of Lake Washington contained bacteria which appeared identical to 37W, we were not successful in isolating these in pure culture. Enrichments from the epilimnion of both lakes did not become turbid even after 6 weeks of incubation.

General characterization of isolates 37W and H4-14. As is shown in Table 1 and Fig. 1a and c, the cells of isolates 37W and H4-14 were both gram-negative, pleomorphic rods, with H4-14 cells slightly larger. These organisms were distinguishable on the basis of colony type; isolate 37W was white on agar plates, whereas isolate H4-14 was mucoid and light to bright yellow on agar plates. Isolate 37W was motile, and isolate H4-14 was not.

The two isolates contained DNA with a G+C content of 66% (for 37W) and 58% (for H4-14). The optimum growth temperature for cells grown in NMS was 30°C for 37W and 32°C for H4-14. For H4-14, the optimum growth temperature in NFMS medium was 23°C. Isolate 37W required a yeast extract supplement (0.005%, wt/vol) for growth on all substrates. This requirement could not be replaced by supplementation with vitamins or amino acids.

Fine structure. Figure 1b and d shows elec-

TABLE 1. *Characteristics of two facultative methane-oxidizing bacteria*

Property	37W	H4-14
Colonial characteristics	White	Yellow, mucoid
Gram reaction	Negative	Negative
Shape	Pleomorphic rods	Pleomorphic rods
Size	0.4 by 0.9 μm	0.5 by 1.2 μm
Motility	+	—
G+C content	66%	58%
Temperature optimum		
NMS	30°C	32°C
NFMS	ND ^a	23°C
Generation time ^b		
Methane		
NMS	45	17
NFMS	ND ^a	19
Nutrient broth	4	5

^a ND, Not done; isolate 37W requires a yeast extract supplement for growth.

^b Hours per generation at optimum temperature.

tron micrographs of thin sections of isolates H4-14 and 37W, respectively. In both organisms, cells grown on methane showed extensive intracytoplasmic membrane pairs arranged at the periphery. This membrane arrangement has been previously designated type II (2). Thin sections made from heterotrophically grown cells did not show such membranes.

Utilization of alternate sources of carbon and energy. Table 2 shows the relative growth of isolates H4-14 and 37W on agar plates containing various substrates as compared to background growth on agar plates lacking a substrate. Plates were scored in all cases after incubation for 1 week. In those cases where no growth was apparent after 1 week, incubation was continued for an additional 2 weeks. Both organisms grew on methane and a variety of organic compounds including tricarboxylic acid intermediates and sugars. Growth also occurred on complex media, such as nutrient agar and Casamino Acids. The organisms did not show the same pattern of utilization. Isolate 37W grew on galactose and acetate, whereas isolate H4-14 did not. Isolate H4-14 grew on ethanol and sucrose, but isolate 37W would not. Neither organism grew on propanol.

Oxidation experiments with whole-cell suspensions. Table 3 shows the rates of oxidation of various compounds by cell suspensions of H4-14 and 37W grown on different carbon sources. When the organisms were grown on a combination of methane plus a heterotrophic substrate, the only compounds tested were methane and the heterotrophic substrate.

Methane-grown H4-14 and 37W cells oxidized methane, alkenes (ethylene and propylene), secondary butanol, and succinate. Methane-grown

H4-14 cells did not oxidize acetate, α -ketoglutarate, or glucose but oxidized primary alcohols (methanol, ethanol, and propanol). Methane-grown 37W cells did not oxidize primary alcohols, α -ketoglutarate, or acetate, but did oxidize glucose. Alkene oxidation in both organisms was probably carried out by the methane monooxygenase, which has been shown to oxidize alkenes in obligate methanotrophs (21, 26).

Ethanol-grown H4-14 cells showed either non-detectable or lower oxidation rates of methane, alkenes, methanol, and propanol. Acetate, α -ketoglutarate, and glucose were oxidized at higher rates. Succinate-grown 37W cells showed very low rates of oxidation in general, oxidizing only succinate, α -ketoglutarate, and glucose. All other compounds listed either were not oxidized or were oxidized at quite low rates.

Cells of H4-14 grown on a combination of methane plus ethanol oxidized both methane and ethanol at high rates. However, 37W cells grown on methane plus succinate oxidized methane quite poorly and oxidized succinate at a rate intermediate between methane-grown and succinate-grown cells.

Enzymes of C-1 assimilation. Table 4 lists the results of enzyme assays from cell-free extracts of 37W and H4-14. The oxidation enzyme, methanol dehydrogenase, as well as all of the key enzymes for the serine pathway for fixation of formaldehyde (hydroxypyruvate reductase, serine-glyoxylate aminotransferase, glycerate kinase, phosphoenolpyruvate carboxylase, serine transhydroxymethylase, and malyl CoA lyase) were found at high levels in both types of cells grown on methane and at low or nondetectable levels in cells grown on a heterotrophic substrate or on a heterotrophic substrate plus methane. The key enzyme in the ribulose 5-phosphate pathway for fixation of formaldehyde, hexulose phosphate synthase, was not detected, although it was detected in a control assay (using an extract of a methanol utilizer which is known to contain the ribulose monophosphate pathway).

Phosphoenolpyruvate carboxylase activity in methane-grown cells was not stimulated by acetyl CoA in either H4-14 or 37W. However, when the organisms were grown on their respective heterotrophic carbon sources, the phosphoenolpyruvate carboxylase activity from each was stimulated by acetyl CoA.

H4-14 contained a glyoxylate-stimulated serine transhydroxymethylase activity during growth on methane, but not during growth on ethanol. In 37W, however, serine transhydroxymethylase activity was not stimulated significantly by glyoxylate in extracts of either methane- or succinate-grown cells. This enzyme was

FIG. 1. Photomicrographs of methane-grown cells of isolates H4-14 and 37W. (a) Phase micrograph of isolate H4-14; (b) electron micrograph of isolate H4-14; (c) phase micrograph of isolate 37W; (d) electron micrograph of isolate 37W. Bar = 4 μm (a, c) or 0.5 μm (b, d).

assayed by using two different techniques (see Materials and Methods). Glyoxylate activation could not be detected by using the coupled assay (10) since the coupling enzyme (methylene tetrahydrofolate dehydrogenase) was strongly inhibited by glyoxylate. Therefore, the radioactive assay (17) was used for all determinations of this activity.

The nicotinamide adenine dinucleotide-linked secondary alcohol dehydrogenase and isocitrate lyase activities were not detected in either 37W or H4-14.

Alpha-ketoglutarate dehydrogenase was detected at low specific activities in methane-grown cells of both H4-14 and 37W. The specific activity increased in cells of both organisms

grown on their respective heterotrophic medium, or in the presence of methane plus the heterotrophic substrate.

Nitrogen fixation assays. Isolate 37W would not grow in completely nitrogen-free medium, since a supplement of yeast extract (0.005% wt/vol) was always required for growth. Isolate H4-14 grew well in NFMS in the presence of methane, but the optimal growth temperature was significantly lower than for growth in the presence of nitrate (Table 1). When acetylene reduction was measured in the presence of methanol in H4-14 cells grown on methane (NFMS medium), ethylene was produced at a rate of 31.2 nmol/min per mg of protein. No ethylene was produced from acetylene in cells grown on methane in NMS medium. Isolate H4-14 was unable to grow in NFMS medium containing ethanol, even under reduced oxygen tensions.

DISCUSSION

This paper describes the characterization of two new facultative methanotrophs, isolates H4-14 and 37W. Both organisms utilize methane and a variety of other organic compounds as sole sources of carbon and energy.

Electron micrographs demonstrated the presence of type II membranes in the methane-grown cells of both isolates. Like other methanotrophs with type II membrane systems (3), both isolates contain α -ketoglutarate dehydrogenase and appear to assimilate carbon via the isocitrate lyase-serine pathway.

Methane oxidation, the internal membrane system, and the serine pathway appear to be induced during growth on methane in both cases. In 37W, the lack of methane oxidation

TABLE 2. Utilization of carbon and energy sources by isolates 37W and H4-14

Carbon and energy source	Organism ^a	
	37W	H4-14
Methane	++	++
Ethanol	—	++
Glucose	±	+
Succinate	++	+
Xylose	+	+
Galactose	+	—
Propanol	—	—
Sucrose	—	±
Lactate	±	±
Malate	±	+
Fructose	+	±
Acetate	±	—
Methanol	±	±
Nutrient agar	+++	+++
Casamino Acids	++	+++

^a +, Growth; —, no growth; ±, slight growth.

TABLE 3. Oxidation of various compounds by whole-cell suspensions of isolates H4-14 and 37W grown on different carbon sources^a

Substrate	Rate of oxidation (nmol of O ₂ consumed per min per mg of protein)					
	H4-14 grown on:			37W grown on:		
	Methane	Methane + ethanol	Ethanol	Methane	Methane + succinate	Succinate
Methane	413	319	42	305	5	0 ^b
Ethylene	73		15	118		5
Propylene	24		0 ^b	361		0 ^b
Methanol	226		0 ^b	0 ^b		1
Ethanol	79	188	89	0 ^b		5
Propanol	179		90	0 ^b		6
2-Butanol	49		49	25		0 ^b
Acetate	0 ^b		160	0 ^b		3
Succinate	43		45	114	68	37
α -Ketoglutarate	0 ^b		109	0 ^b		15
Glucose	0 ^b		119	37		11

^a All values are the average of two or more experiments. Values agreed $\pm 15\%$.

^b Not detectable.

TABLE 4. Enzymatic activities in extracts of isolates H4-14 and 37W grown on different carbon sources^a

Enzyme	Activity ($\mu\text{mol}/\text{min}$ per mg of protein)					
	37W grown on:			H4-14 grown on:		
	Methane	Succinate + methane	Succinate	Methane	Methane + ethanol	Ethanol
Hydroxypyruvate reductase	0.412	0.013	0.007	0.794	0.003	0.011
Serine-glyoxylate aminotransferase	0.269	0 ^b	0.004	0.087	0.001	0.003
Glycerate kinase	0.057	0 ^b	0 ^b	0.056	0.009	0.001
Phosphoenolpyruvate carboxylase						
– Acetyl CoA	0.12	0 ^b	0.003	0.065	0.006	0.003
+ Acetyl CoA ^c	0.12	0.03	0.013	0.065	0.006	0.010
Serine transhydroxymethylase						
– Glyoxylate	0.018	0.006	0.007	0.013	0 ^b	0.003
+ Glyoxylate ^c	0.020	0.008	0.003	0.051	0 ^b	0.004
Malyl CoA lyase	0.212	0.004	0.001	0.092	0 ^b	0.004
Methanol dehydrogenase	0.039	0.003	0.007	0.050	0.002	0.007
Secondary alcohol dehydrogenase	0 ^b	NT ^d	NT	0 ^b	NT	NT
α -Ketoglutarate dehydrogenase	0.004	0.015	0.017	0.008	0.060	0.064
Hexulose phosphate synthase	0 ^b	NT	NT	0 ^b	NT	NT
Isocitrate lyase	0 ^b	NT	0 ^b	0 ^b	NT	0 ^b

^a All values are the average of two or more experiments. Values agreed $\pm 15\%$.^b Not detectable.^c 2.5 mM.^d NT, Not tested.

and the low levels of serine pathway enzymes in cells grown on a combination of methane plus succinate indicate, first, that these enzymes are repressed during growth on succinate, and, second, that methane is unable to alleviate this repression.

In H4-14 however, cells grown on ethanol plus methane oxidized methane at a high rate and had low levels of serine pathway enzymes. This suggests that the methane oxidation system is not repressible in this isolate but that the serine pathway is repressible. The methane oxidation system in H4-14 is not constitutive, since it was observed at low levels in ethanol-grown cells.

These two isolates both contain an acetyl CoA-independent phosphoenolpyruvate carboxylase activity during growth on methane, but an acetyl CoA-dependent activity during growth on heterotrophic substrates. Such results could be explained by the presence of two different enzymes, one which served a major assimilatory function and one which served an anaplerotic function. Evidence for such isoenzymes has been reported in methanol utilizers (16) and observed in *Methylobacterium organophilum* XX (M. O'Connor, unpublished results).

H4-14 contained a glyoxylate-stimulated serine transhydroxymethylase activity in methane-grown cells, but not in cells grown on ethanol. These results are consistent with the presence

of isoenzymes for this activity also. Two such isoenzymes have been purified from *M. organophilum* XX (17). The serine transhydroxymethylase from methane-grown 37W cells did not show this stimulation.

Although whole cells oxidized secondary butanol, the secondary alcohol dehydrogenase activity described by Hou et al. (11) was not detected in either isolate. This unusual activity may therefore not be as widespread in methanotrophs as suggested by Hou and co-workers.

In terms of presumptive classification, isolates H4-14 and 37W are similar to the other facultative methanotrophs *M. organophilum* XX (22, 23) and *M. organophilum* R₆ (20) in that they have the ability to use both methane and more complex compounds as sole sources of carbon and energy. Before the work of Patt et al. (22), this was not considered to be a feature of methanotrophs (28). However, the success of three separate investigations in isolating facultative methanotrophs without selective pressure toward growth on alternate carbon sources suggests that these bacteria may be a common element in methanotrophic populations of freshwater lakes and soils.

Table 5 compares the four facultative methanotrophs in terms of several features. It can be seen that there are distinct differences among them: all four organisms showed different pig-

TABLE 5. Comparison of four facultative methanotrophs

Feature	<i>M. organophilum</i> XX ^a	<i>M. organophilum</i> R ₆ ^b	H4-14	37W
Color on agar plates	Pink	Brown	Light to dark yellow	White
Size	1 by 1.5 μ m	0.6 by 2 μ m	0.5 by 1.2 μ m	0.4 by 0.9 μ m
Motility	+	+	—	+
Growth on substrate ^c				
Ethanol	—	+	+	—
Galactose	+	—	—	+
Lactose	+	—	±	±
Acetate	+	+	±	±
Sucrose	+	—	±	—
Lactose	+	—	±	±
Methanol	+	+	—	—
α -Ketoglutarate dehydrogenase	+	+	+	+
G+C content	66%	ND ^d	58%	66%
Resting stage	None	Exposure	None	None

^a Reference 22.^b Reference 20.^c All four organisms grew on methane, succinate, and nutrient agar with varying confluency. None utilized propanol.^d ND, Not done.

mentation on agar plates and *M. organophilum* XX cells were larger in size than the others. The four organisms displayed a variety of abilities to utilize carbon compounds. *M. organophilum* XX and isolate 37W had similar G+C values (66%). The percent G+C of isolate H4-14 was significantly lower, suggesting that this organism may not be closely related to the other two. This DNA base ratio is also lower than that reported for obligate type II methanotrophs (3). A value for the G+C was not reported for *M. organophilum* R₆.

A resting stage was seen in *M. organophilum* R₆ in the form of an exospore, whereas no such resting stages were observed with the other three isolates.

We believe that H4-14 and 37W belong to the genus *Methylobacterium*, since they are facultative methanotrophs. However, there are sufficient differences between these two and the two previously described isolates such that it is recommended that isolates H4-14 and 37W each be further classified into new and separate species. The following names are proposed.

H4-14: *Methylobacterium ethanolicum* sp. nov. (eth an ol' i cum). I.S.V. noun *ethanol* two-carbon alcohol; M.L. adjective *ethanolicum*, intended to refer to the preferred heterotrophic substrate.

Nonmotile, pleomorphic gram-negative rods measuring 0.5 by 1.2 μ m.

Colony characteristics: light to bright yellow, circular in form with entire margins; mucoid.

37W: *Methylobacterium hypolimneticum* sp. nov. (hy po lim net' i cum). Gr. adjective *hypo* under; Gr. adjective *limnetic* relating to or inhabiting the pelagic part of the freshwater; N.L. adjective *hypolimneticum* referring to the hypolimnion where this organism was found.

Motile, pleomorphic gram-negative rods measuring 0.4 by 0.9 μ m.

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LITERATURE CITED

- Blackmore, M. A., and J. R. Quayle. 1970. Microbial growth on oxalate by a route not involving glyoxylate carboligase. *Biochem. J.* 118:53-59.
- Canovas, J. L., and H. L. Kornberg. 1969. Phosphoenolpyruvate carboxylase from *Escherichia coli*. *Methods Enzymol.* 13:288-292.
- Colby, J., H. Dalton, and R. Whittenbury. 1969. Biological and biochemical aspects of microbial growth on C₁ compounds. *Annu. Rev. Microbiol.* 33:481-517.
- Dahl, J. S., R. J. Mehta, and D. S. Hoare. 1972. New obligate methylotroph. *J. Bacteriol.* 109:916-921.
- Dalton, H., and R. Whittenbury. 1976. The acetylene reduction technique as an assay for nitrogenase activity in the methane oxidizing bacterium, *Methylococcus capsulatus* strain Bath. *Arch. Microbiol.* 109:147-151.
- Davies, S. L., and R. Whittenbury. 1970. Fine structure of methane and other hydrocarbon utilizing bacteria. *J. Gen. Microbiol.* 61:227-232.
- Dixon, G. H., and H. L. Kornberg. 1959. Assay methods for the key enzymes of the glyoxylate cycle. *Biochem. J.* 72:36.
- Gibson, D. N., and J. D. Ogden. 1979. A rapid method for purifying bacterial DNA. *J. Appl. Bacteriol.* 46:421-422.
- Guest, J. R., and I. T. Creaghan. 1973. Gene-protein relationships of the α -keto acid dehydrogenase complexes of *Escherichia coli* K12. Isolation and characterization of lipoamide dehydrogenase mutants. *J. Gen. Microbiol.* 75:197-210.
- Heptinstall, J., and J. R. Quayle. 1970. Pathways leading to and from serine during growth of *Pseudomonas* AM1 on C-1 compounds or succinate. *Biochem. J.* 117:563-572.
- Hou, C. T., R. Patel, A. I. Laskin, N. Barnabe, and I. Marczak. 1979. Microbial oxidation of gaseous hydrocarbons: production of methyl ketones from their corresponding secondary alcohols by methane- and methanol-grown microbes. *Appl. Environ. Microbiol.* 38:135-142.
- Jannasch, H. W., and W. S. Moddux. 1967. A note on bacteriological sampling in seawater. *J. Mar. Res.* 25:185-189.
- Large, P. J., and J. R. Quayle. 1963. Microbial growth on C₁ compounds. 5. Enzyme activities in extracts of *Pseudomonas* AML. *Biochem. J.* 87:386-395.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
- Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* 5:109-118.

16. Newaz, S. S., and L. B. Hersh. 1978. Reduced nicotinamide adenine dinucleotide-activated phosphoenolpyruvate carboxylase in *Pseudomonas* MA: potential regulation between carbon assimilation and energy production. *J. Bacteriol.* **124**:825-833.
17. O'Connor, M. L., and R. S. Hanson. 1975. Serine transhydroxymethylase isoenzymes from a facultative methylotroph. *J. Bacteriol.* **124**:985-996.
18. O'Connor, M. L., and R. S. Hanson. 1977. Enzyme regulation in *Methylobacterium organophilum*. *J. Gen. Microbiol.* **101**:327-332.
19. O'Connor, M. L., and R. S. Hanson. 1978. Linkage relationships between mutants of *Methylobacterium organophilum* impaired in their ability to grow on one-carbon compounds. *J. Gen. Microbiol.* **104**:105-111.
20. Patel, R. N., C. T. Hou, and A. Felix. 1978. Microbial oxidation of methane and methanol: isolation of methane-utilizing bacteria and characterization of a facultative methane-utilizing isolate. *J. Bacteriol.* **136**:352-358.
21. Patel, R. N., C. T. Hou, A. I. Laskin, A. Felix, and P. Derelanko. 1979. Microbial oxidation of gaseous hydrocarbons. II. Hydroxylation of alkenes by cell-free particulate fractions of methane-utilizing bacteria. *J. Bacteriol.* **139**:675-679.
22. Patt, T. E., G. C. Cole, J. Bland, and R. S. Hanson. 1974. Isolation and characterization of bacteria that grow on methane and organic compounds as sole sources of carbon and energy. *J. Bacteriol.* **120**:955-964.
23. Patt, T. E., G. C. Cole, and R. S. Hanson. 1976. *Methylobacterium*, a new genus of facultative methylotrophic bacteria. *Int. J. Syst. Bacteriol.* **26**:226-229.
24. Salem, A. R., A. J. Hacking, and J. R. Quayle. 1973. Cleavage of malylCoA into acetyl coenzyme A and glyoxylate by *Pseudomonas* AM1 and other C-1 utilizing bacteria. *Biochem. J.* **136**:89-96.
25. Sohngen, N. L. 1906. Über Bakterien, welche Mathen als Kohlenstoffnafrung und Energiequelle gebrauchen. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2* **15**:513-517.
26. Stirling, D. I., J. Colby, and H. Dalton. 1979. A comparison of the substrate and electron-donor specificities of the methane-oxidizing bacteria. *Biochem. J.* **177**:361-364.
27. Whittenbury, R. K., S. L. Davies, and J. E. Davey. 1970. Exospores and cysts formed by methane-utilizing bacteria. *J. Gen. Microbiol.* **61**:219-227.
28. Whittenbury, R. K., K. C. Phillips, and J. F. Wilkinson. 1970. Enrichment, isolation and some properties of methane utilizing bacteria. *J. Gen. Microbiol.* **61**:205-218.