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Acetobacter methanolicus sp. nov., an Acidophilic Facultatively Methylotrophic Bacterium

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A new species, *Acetobacter methanolicus*, is described. The strains investigated were isolated from sludge and from a yeast fermentation process in which methanol was the sole source of carbon and energy. A total of about 140 phenotypic features were tested. The strains proved to be acidophilic and facultatively methylotrophic, and they differed from other *Acetobacter* species by growing well on methanol, glucose, gluconate, 2,3-butanediol, and caproic acid as sole sources of carbon and energy. Ethanol was "overoxidized" only at initial concentrations of <0.5%. Lactate was oxidized very weakly, but it was not utilized as a sole carbon source for growth. Yeast extract or pantothenic acid was essential for growth. The specific epithet of the proposed new species refers to its isolation from media in which methanol was the sole source of carbon. The deoxyribonucleic acid base composition of type strain MB58 (= IMET 10945) is 62.3 mol% guanine plus cytosine.

Methylotrophic bacteria are bacteria which can grow aerobically on reduced carbon compounds with one or more carbon atoms but without C-C bonds (e.g., methane, methanol, methylamine, and other compounds). In two recent valid publications this property was used to name the following bacterial species which grew well on methanol in the neutral pH range: *Methylobacillus glycogenes* Yordy and Weaver 1977, a gram-negative obligately methylotrophic rod-shaped organism (42), and *Methylobacterium organophilum* Patt, Cole and Hanson 1976 (31). Based on the results of their investigations in 1982 (19), Green and Bousfield emended the description of the genus *Methylobacterium* (20). These authors proposed that *Methylobacterium* should include all gram-negative, pink-pigmented, facultatively methylotrophic bacteria.

We succeeded in isolating several bacterial strains from sludge and from a septic methanol yeast process which resembled each other very closely and proved to be gram-negative, facultatively methylotrophic rod-shaped organisms that grow aerobically in the acid pH range; these strains were designated the MB strains. Some publications on these strains, especially strain MB58^T (T = type strain), have appeared which deal with their growth, with the incorporation of methanol into cells via the hexulose phosphate pathway (5–8, 26), with their cytochrome spectra (9, 33), and with their ability to oxidize several carbon compounds (34).

It has been reported that these strains, because of their growth optima at pH 4.0 to 4.5 and their capacities to oxidize ethanol to acetate and glucose to gluconate in acid media and to form acids from a number of sugars and alcohols, behave as acetic bacteria (5, 26, 33, 34). In addition, the growth of a few *Acetobacter pasteurianus* strains on methanol was recently reported by Gosselé et al. (17). In this paper we define the characteristics of, discuss the taxonomic position of, and propose a name for the facultatively methylotrophic acetic acid bacteria which we isolated.

MATERIALS AND METHODS

Bacterial strains and media. The strains which we studied and their sources are listed in Table 1. These strains were grown and maintained on glucose-yeast extract agar (24) and potato-glycerol agar (1). In addition, the MB strains were grown and maintained on the following media: glucose agar (pH 4.0) containing (per liter) 3 g of $(NH_4)_2SO_4$, 1 g of KH_2PO_4, 0.16 g of K_2PO_4, 0.7 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.4 g of $Ca(NO_3)_2 \cdot 4H_2O$, 20 g of glucose, 5 g of yeast extract, and 20 g of agar; and standard agar (pH 4.0 or 5.4) containing 1% methanol, 31 or 62 mg of P per liter (1 or 2 ml of a solution containing 68.05 g of KH_2PO_4 per liter and 87.09 g of K_2HPO_4 per liter), 200 mg of N per liter (5 ml of a solution containing 152.28 g of NH_4Cl per liter or 10 ml of a solution containing 121.3 g of $NaNO_3$ per liter), 1 mg of Ca per liter (1 ml of a solution containing 5.47 g of $CaCl_2 \cdot 6H_2O$ per liter), 0.5 g or 1.0 g of yeast extract per liter, 20 g of agar (Difco Laboratories, Detroit, Mich.) per liter, and 1 ml of a trace element solution (71.2 g of $MgSO_4 \cdot 7H_2O$ per liter, 0.44 g of $ZnSO_4 \cdot 7H_2O$ per liter, 0.812 g of $MnSO_4 \cdot 4H_2O$ per liter, 0.785 g of $CuSO_4 \cdot 5H_2O$ per liter, 0.252 g of $Na_2MoO_4 \cdot 2H_2O$ per liter, 4.98 g of $FeSO_4 \cdot 7H_2O$ per liter) per liter.

The same medium but without agar was used as the standard medium for the experiments performed in shakers. The temperature for growth was 30°C; the strains were maintained at 2 to 4°C and were transferred to new media every 4 weeks (agar cultures) or every 6 weeks (liquid cultures). Growth in liquid media was assessed by measuring the turbidity with a Spekol photometer (VEB Carl Zeiss, Jena, German Democratic Republic); growth was positive when the biomass increased according to the amount of carbon source added.

Nutritional, physiological, and biochemical tests. A total of about 140 phenotypic features were tested. The methods of Leifson (25) and Carr (11) were used to detect the overoxidation of ethanol and the oxidation of lactate to CO_2 and H_2O. Lactate oxidation was also tested polarographically by using the method of Asperger and Aurich (4). The oxidation of glutamic acid by resting cells was studied by using the same method. The medium of Asai et al. (3) was used to detect acid production from different carbon compounds; the tests were finished after 7 days of incubation at 30°C. Growth on methanol was studied on methanol-containing standard medium (or agar) and in Hoyer medium as modified by Frateur (14), using methanol concentrations of 1% as well as 0.3% as described by Gosselé et al. (16). The oxidation products of methanol (formate) formed in shakers were determined by

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TABLE 1. Designations and sources of *Acetobacter* strains studied

Species	Strain[a]	Source[a]	Comments
A. aceti	NCIB $8621t_1^T$	De Ley	
A. pasteurianus	LMD $22.1t_1^T$	De Ley	
	ATCC 12876	ATCC	Former type strain of *A. aceti* subsp. *orleanensis*[b]
	Martin 1	De Ley	For further details
	LMG 76.10	De Ley	see references 16
	NCPPB 461	De Ley	and 17
	NCPPB 462	De Ley	
	NCPPB 463	De Ley	
	NCIB 8620	De Ley	Former type strain of *A. pasteurianus* subsp. *lovaniensis*[b]
A. methanolicus	MB57	IBT	Isolated from sludge
	$MB58^T$	IBT	IMET 10945^T, isolated from septic methanol-yeast process
	MB60	IBT	Isolated from sludge

[a] De Ley, J. De Ley, Laboratorium voor Microbiologie en microbiele Genetica (LMG), Gent, Belgium; ATCC, American Type Culture Collection, Rockville, Md.; IBT, Institut für Biotechnologie der Akademie der Wissenschaften der Deutsche Demokratische Republik, Leipzig, German Democratic Republic; IMET, Institut für Mikrobiologie und experimentelle Therapie der Akademie der Wissenschaften der Deutsche Demokratische Republik; Jena, German Democratic Republic; LMD; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NCPPB, National Collection of Plant Pathogenic Bacteria.

[b] See references 17 and 32.

using the method of Lang and Lang (23). Modified Hoyer media were also used to test the utilization of 3% (vol/vol) glucose, 3% (vol/vol) mannitol, and 3% (vol/vol) ethanol in the presence of ammonium (pH 5.4). Growth on more than 90 carbon compounds (as sole carbon sources) was examined by using auxanograms or, if necessary, in shakers. The basic agar medium used for the auxanograms contained (per liter) 5.0 g of $(NH_4)_2SO_4$, 1.0 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of yeast extract (Difco), and 20 g of agar (Difco); the initial pH was 5.4. Cultures that were 48 h old were suspended and mixed with liquefied basic agar, and then the mixture was poured into petri dishes. A few particles of the compounds to be tested were put on the agar surface (if the compound was a liquid, 1 drop was put into a punch hole). The dishes were incubated at 30°C, and increasing turbidity in the agar around the test compound indicated growth. Ketogenesis from glycerol and from mannitol and sorbitol and the formation of γ-pyrones were determined by using the methods of Yamada et al. (41). The formation of pigments was tested on GYC medium (16); to determine the formation of H_2S, indole, catalase, and gelatinase and the reduction of nitrates, the tests of Swings et al. (36) were used. The method of Gosselè et al. (15) was used to detect 2-ketogluconic, 5-ketogluconic, and 2,5-diketogluconic acids. Ubiquinones were determined by thin-layer chromatography (M. Worbs, Ph.D. thesis, Institute für Biotechnologie der Akademie der Wissenschaften der Deutsche Demokratische Republik, Leipzig, 1985). The utilization of NH_4^+ or NO_3^- as a sole nitrogen source for growth was studied in the presence of methanol, glucose, ethanol, and mannitol in Hoyer medium (NH_4^+) or in standard medium (NH_4^+ or NO_3^-). Microscopic features of the cells were studied by bright-field and phase-contrast microscopy. Gram-stained cells were prepared by using crystal violet and the method of Drews (13). Specimens for flagellum staining were fixed with the vapor of a 2% OsO_4 solution (13), and the flagella were stained by the Loeffler method (21). The formation of acetylmethylcarbinol was tested at 30 and 37°C in a medium containing 2% sodium DL-lactate and was detected as described by Gosselé et al. (16).

RESULTS

After incubation at 30°C for 3 or 4 days on standard agar containing methanol, the colonies of strains MB57, $MB58^T$, and MB60 were about 1 mm in diameter, slightly convex, circular with entire margins, smooth, and weakly glistening, had a moist and smeary consistency, and were white to pale yellow (strain MB60 was weakly pink). The cells of the strains were ellipsoidal to rod shaped, (0.6 to 0.8 by 1.0 to 1.8 μm). They occurred singly, in pairs, and sometimes in short chains; they were gram negative and did not form endospores. Young cells were motile, and their flagellation seemed to be peritrichous; polarly inserted flagella have never been detected. The optimum conditions for growth were 30 to 32°C and pH 4.0 to 4.5. Physiologically, strains MB57, $MB58^T$, and MB60 reacted alike. Their metabolism was respiratory, never fermentative; they were obligately aerobic, catalase positive, and gelatinase negative and did not form H_2S, indole, or nitrite from nitrate. They required about 0.05% yeast extract or about 0.00025% pantothenic acid as a growth factor. The mean doubling time of the cells on methanol- or glucose-containing media was about 4 h.

At an initial pH of <4.5 ethanol was oxidized to acetic acid, but ethanol was endoxidized (overoxidized) to CO_2 and H_2O only at initial concentrations of <0.5%. In contrast to the type and reference strains, the MB strains did not grow on lactate, but they were able to oxidize lactate very slowly. The oxidation of lactate was detected polarographically and in Leifson lactate medium, which was weakly alkalized. On the $CaCO_3$-containing agar media of Carr (11), irisation was observed with ethanol, but not with lactate.

The strains tested showed weak ketogenesis from glycerol, but not from mannitol or sorbitol. Glucose was strongly oxidized to gluconate, but 2-, 5-, and 2,5-ketogluconic acids, γ-pyrones, and brown pigments were not formed. Resting cells of the strains were able to oxidize glutamic acid. Ammonium and nitrate were utilized as sole sources of nitrogen, but amino acids were not (we tested L-alanine, L-asparagine, L-glutamic acid, L-glutamine, L-glycine, L-lysine, L-proline, L-threonine, and L-tryptophan by the method of Gosselé et al. [18]).

The deoxyribonucleic acid (DNA) base composition of strain $MB58^T$ was 62.3 mol% guanine plus cytosine (thermal denaturation method), as determined by W. Boeckel (Institut für Mikrobiologie und Experimentelle Therapie der Akademie der Deutsche Demokratische Republik, Jena, German Democratic Republic), who used the methods of Marmur and Doty (27, 28). Table 2 compares some characteristics of the MB strains with characteristics of the genera *Gluconobacter* and *Acetobacter*, as given by De Ley et al. (12). These characteristics show obvious conformity with the genus *Acetobacter*. DNA-ribosomal ribonucleic acid hybridizations confirmed that the MB strains belong to the genus *Acetobacter* (J. De Ley and M. Gillis, Gent, Belgium, personal communication). Experiments dealing with the utilization of more than 90 carbon compounds (e.g., sugars, sugar alcohols, organic acids, and their salts) as sole sources of carbon gave the results described below. None of the strains listed in Table 1 grew on methane, formaldehyde, formate, L-arabinose, D-arabinose, D-xylose, D-ribose, D-

TABLE 2. Characteristics of *Gluconobacter* and *Acetobacter* compared with those of the MB strains[a]

Characteristic	*Gluconobacter*	*Acetobacter*	MB strains
Flagellation of motile strains:			
Polar	+	−	−
Peritrichous	−	+	+
Overoxidation of ethanol	−	+	+
Oxidation of DL-lactate to CO_2 and H_2O	−	+	(+)
Oxidation of acetate to CO_2 and H_2O	−	+	+
Oxidation of amino acids by resting cells	−	+	+
Ketogenesis	+	D	(+)
Formation of brown water-soluble pigments on GYC agar	−	D	−
Growth factors required	+	D	+
Products formed from D-glucose:			
2-Ketogluconic acid	+	D	−
5-Ketogluconic acid	+	D	−
2,5-Diketogluconic acid	D	D	−
Acetylmethylcarbinol (Voges-Proskauer)	D	D	−
Type of ubiquinone formed:			
Q_9	−	D	−
Q_{10}	+	D	+
Acid produced from:			
D-Arabinose	+	−	+
i-Inositol	D	−	−
Maltose	D	−	−
D-Fructose	+	−	−
Carbon sources for growth:			
Acetate	−	D	(+)
Lactate	−	D	−
Guanine-plus-cytosine content of DNA (mol%)	57–64	51–65	62.3

[a] Data for *Gluconobacter* and *Acetobacter* from reference 12.
[b] +, Positive; (+), weakly positive; −, negative; D, different reactions in different taxa.

mannose, D-galactose, L-rhamnose, L-sorbose, sucrose, lactose, maltose, D-cellobiose, melibiose, D-raffinose, D-melezitose, starch, cellulose, D-salicin, esculin, arbutin, N-acetylglucosamine, D-galactosamine, 2-ketogluconate, inositol, adonitol, dulcitol, L-arabitol, sodium oxalate, oxalic acid, sodium succinate, adipinic acid, glutaric acid, α-ketoglutarate, sodium fumarate, potassium tartrate, citric acid, sodium citrate, phenol, sodium benzoate, *p*-aminobenzoic acid, 3-hydroxylbenzoic acid, methylamine hydrochloride, creatine, creatinine, anthranilic acid, ammonium salicylate, nicotinic acid, glycine, L- or DL-α-alanine, β-alanine, L- or DL-valine, L- or DL-leucine, L-isoleucine, L- or DL-serine, L- or DL-cysteine, L- or DL-methionine, L-aspartic acid, L-asparagine, L-aspartate, L-glutamic acid, L-glutamine, L-glutamate, L- or DL-phenylalanine, L- or DL-proline, L- or DL-tryptophan, L-histidine, L-citrulline, DL-norleucine, L- or DL-cystine, L- or DL-threonine, L- or DL-arginine, L-lysine, L-ornithine hydrochloride, L- or DL-tyrosine, or DL-thyroxine. The utilization of other compounds by the strains varied (Tables 3 and 4).

Characteristics which differentiate the species of the genus *Acetobacter* (12) are compared with the characteristics of the MB strains in Table 3. It is obvious that the MB strains are very similar to *A. pasteurianus*. This agrees with the protein gel electropherograms made from our strains and compared with numerous protein profiles obtained from many *Gluconobacter* and *Acetobacter* strains; the electropherograms of the MB strains did not resemble at all the electropherograms of *Gluconobacter* spp., *Acetobacter liquefaciens*, *Acetobacter aceti*, or *Acetobacter hansenii* (De Ley and Gillis, personal communication).

It seemed that the MB strains might belong to *A. pasteurianus*, which is genetically very heterogeneous and includes, among other organisms, the methanol-utilizing strains described by Gosselé et al. (17). Consequently, the MB strains were compared with the type strain and several reference strains of *A. pasteurianus*. However, Table 4

TABLE 3. Characteristics that differentiate the species of the genus *Acetobacter* and the MB strains[a]

Characteristic	*A. aceti*	*A. liquefaciens*	*A. pasteurianus*	*A. hansenii*	*A. methanolicus* (MB strains)
Formation of:					
Water-soluble brown pigments on GYC agar	−	+	−	−	−
γ-Pyrones from D-glucose	−	d	−	−	−
γ-Pyrones from D-fructose	−	+	−	−	−
5-Ketogluconic acid from D-glucose	+	d	−	d	−
2,5-Diketogluconic acid from D-glucose	−	+	−	−	−
Ketogenesis from:					
Glycerol	+	+	−	+	(+)
Sorbitol	+	+	−	+	−
Mannitol	d	+	−	+	−
Growth on carbon sources:					
Ethanol	+	+	d	−	(+)
Dulcitol	−	−	−	d	−
Sodium acetate	+	d	d	−	(+)
Growth on L-amino acids in the presence of D-mannitol as a carbon source:					
L-Glycine, L-threonine, and L-tryptophan	−	d	−	−	−
L-Asparagine and L-glutamine	d	+	−	+	−
Growth in the presence of 10% ethanol	−	−	d	−	−
Guanine-plus-cytosine content (mol%)[c]	55.9–59.5	62.3–64.6	52.8–62.5	58.1–62.6	62.3[d]

[a] All data except the data for the MB strains obtained from reference 12.
[b] +, Positive in ≧90% of the strains; d, positive in 11 to 89% of the strains; (+), weakly positive; −, negative in ≧90% of the strains.
[c] Determined by the thermal denaturation method.
[d] Value for strain MB58^T.

TABLE 4. Differentiating characteristics for the type and reference strains of *A. aceti*, *A. pasteurianus*, and *A. methanolicus*[a]

Characteristic	*A. aceti*	*A. pasteurianus* strains								*A. methanolicus* strains		
	NCIB 8621t$_1$^{T}	ATCC 12876	NCIB 8620	LMD 22.1t$_1$^{T}	Martin 1	LMG 76.10	NCPPB 461	NCPPB 462	NCPPB 463	MB 57	MB58^T = IMET 10945^T	MB 60
Overoxidation of:												
2% Ethanol	+	+	+	+	+	+	+	+	+	−	−	−
0.5% Ethanol	+	+	+	+	+	+	+	+	+	+	+	+
Oxidation of lactate	+	+	+	+	+	+	+	+	+	(+)	(+)	(+)
Growth on compounds as sole carbon sources												
Methanol	−	−	−	−	−	−	−	−	−	++	++	++
Glucose	(+)	+	−	−	(+)	(+)	−	−	(+)	++	++	++
Sodium-gluconate	(+)	(+)	−	−	(+)	(+)	−	−	(+)	++	++	++
D-Fructose	−	−	−	−	(+)	(+)	−	−	−	(+)	(+)	+
D-Mannitol	(+)	(+)	−	−	−	(+)	−	−	−	−	− or (+)	+
Glycerol	+	+	+	−	+	++	(+)	+	+	++	++	++
Caproic acid	−	−	−	−	−	−	−	−	−	+	+	++
2,3-Butanediol	+	−	−	−	−	−	−	−	(+)	++	++	++
Sodium-DL-lactate	+	+	+	+	+	+	(+)	+	+	−	−	−
Sorbitol	−	−	−	−	(+)	−	−	−	−	−	−	−
m-Erythritol	(+)	(+)	−	−	−	−	−	−	−	−	−	−
Succinic acid	(+)	+	+	(+)	+	(+)	+	+	+	+	+	+
Fumaric acid	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Sodium-pyruvate	(+)	(+)	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Sodium-glyoxylate	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Ketogenesis from:												
Glycerol	+	+	−	−	−	−	(+)	(+)	−	(+)	(+)	(+)
Mannitol	+	+	−	+	−	−	−	−	−	−	−	−
Sorbitol	+	+	−	(+)	−	−	−	−	−	−	−	−
Ubiquinone type (major part)	Q_9	Q_9	Q_9	Q_9	Q_9	Q_9	Q_9	Q_9	Q_9	Q_{10}	Q_{10}	Q_{10}
Formation of acetylmethylcarbinol with sodium DL-lactate at:												
30°C	−	+	+	+	−	+	+	+	+	−	−	−
37°C	−	+	+	+	(+)	+	+	+	+	−	−	−
Acid from:												
Sucrose	+	−	−	−	−	−	−	−	−	−	−	−
Melibiose	D	D	D	D	NT	NT	NT	NT	NT	+	+	+
Mannitol	−	−	−	−	NT	NT	NT	NT	NT	−	−	+
m-Erythritol	+	+	−	−	NT	NT	NT	NT	NT	−	−	−
Sodium-glyoxylate	+	+	+	+	+	+	+	+	+	−	−	−
Methanol	+	+	+	−	−	−	+	+	+	+	+	+

[a] ++, Strongly positive; +, positive; (+), weakly positive; (−), negative to very weakly positive; −, negative; D, delayed positive; NT, not tested.

shows that there were clear distinctions between the MB strains and *A. pasteurianus* strains. This was especially true of growth on methanol, glucose, gluconate, caproic acid, 2,3-butanediol, and lactate, the Voges-Proskauer reaction with 2% sodium DL-lactate, and the ubiquinone type formed. The following features are not shown in Tables 3 and 4: the MB strains formed acid from glucose, galactose, xylose, arabinose, mannose, glycerol, *n*-propanol, i-butanol, *n*-butanol, and amylalcohol; no acid was produced from fructose, sucrose, sorbose, maltose, rhamnose, lactose, raffinose, sorbitol, dulcitol, *m*-erythritol, inulin, starch, salicin, or i-propanol; and growth was weak on ethanol and sodium acetate.

DISCUSSION

Because of the features described above, acidophilic, facultatively methanol-utilizing bacterial strains MB57, MB58^T, and MB60 clearly belong to the genus *Acetobacter* (12). This conclusion is supported by the detection of enzymes of the tricarboxylic acid cycle (5, 6), of cytochrome a_1 (33), and of small amounts of $C_{14:0}$ straight-chain saturated fatty acid (Steudel, Ph.D. thesis, Institut für Technische Chemie der Akademie der Wissenschaften der Deutsche Demokratische Republik, Leipzig, German Democratic Republic, 1982). This fatty acid was detected in addition to the $C_{18:1}$ straight-chain unsaturated fatty acid. As described by Yamada et al. (40), the latter (as a major component of cellular fatty acids) is typical of acetic acid bacteria, and the former is typical of *Acetobacter*.

Within the genus *Acetobacter* it was not possible to assign the MB strains to any previously published species. Thus, we asked (Uhlig, Steudel, and Karbaum, Abstr. IV Int. Conf. Cult. Coll., Brno, Czechoslovakia, 1981) whether these strains should be considered a new species or subspecies of *Acetobacter*. Characteristics which clearly differentiate the MB strains from *A. aceti*, *A. liquefaciens*, and *A. hansenii* are listed in Table 3. More detailed investigations resulted in finding a few striking differences between the MB strains and the strains of *A. pasteurianus* (Table 4).

As the most important differentiating characteristic, the utilization of methanol is discussed first. The oxidation of methanol by strains of acetic acid bacteria has been reported previously by Krehan (22), Bertho (10), Müller (30), and Asai (2), but specific data concerning organisms have not

been published. The same is true for the study of Mimura and Wada (29), who listed the genus *Acetobacter* in a survey of methanol-utilizing bacteria. In addition, whether acidophilic methylotrophic bacterial strain BNS-25 described by Urakami et al. (37) belongs to *Acetobacter* or to *Gluconobacter* cannot be determined from the previously published data.

Concerning the methanol-utilizing *A. pasteurianus* strains of Gosselé et al. (17), we were surprised to find in our experiments on agar and in liquid media at pH 4.0 and 5.4 (with serial transfers) that strains NCIB 8620, NCPPB 461, NCPPB 462, NCPPB 463, Martin 1, and LMG 76.10 did not grow on methanol as a sole carbon source. However, strains MB57, MB58^T, and MB60 grew well; e.g., in shakers with the methanol-containing standard medium the biomass increased from 0.1 g (dry mass) per liter (inoculum) to about 1.5 g (dry mass) per liter within 24 to 48 h. Biochemical findings may explain these results.

The MB strains oxidize methanol, fix it by means of hexulose phosphate synthase, and incorporate it into cells via the fructose biphosphate variant of the hexulose phosphate pathway (5, 7, 9, 26, 34; D. Miethe, Ph.D. thesis, Institut für Technische Chemie der Akademie der Wissenschaften der Deutsche Demokratische Republik, Leipzig, German Democratic Republic, 1977). Babel (5) commented in explanation that phosphofructokinase, which disappears during the assimilation of glucose via the hexose monophosphate pathway, is the key enzyme for assimilation of methanol via the hexulose phosphate pathway in the *Acetobacter* and *Gluconobacter* strains which he tested, because Entner-Doudoroff enzymes and phosphoketolase are missing. Babel detected neither phosphofructokinase in methanol-oxidizing strains NCIB 8620, NCPPB 461, NCPPB 462, and NCPPB 463 not the oxidation of methanol in strains Martin 1 and LMG 76.10, which have phosphofructokinase; consequently, these six strains were unable to grow on methanol as a sole carbon source under the test conditions used.

Another differentiating feature is the oxidation of ethanol. The MB strains oxidized ethanol only at initial concentrations of <0.5%, while the *A. pasteurianus* strains also oxidized ethanol at higher concentrations. Furthermore, the latter preferred ethanol or lactate to glucose for growth, as determined by Swings and De Ley (35). However, the MB strains preferred glucose to ethanol or lactate in the presence of either ammonium or nitrate; lactate was oxidized but was not utilized as a sole carbon source for growth. Moreover and in contrast to the *A. pasteurianus* strains, the MB strains gave a negative Voges-Proskauer reaction with lactate.

Additional differences between the MB strains and the strains of *A. pasteurianus* include good growth of the MB strains on gluconate, caproic acid, and 2,3-butanediol and the ubiquinone type (the *A. pasteurianus* strains have Q_9 as the major ubiquinone [12], whereas the MB strains have Q_{10} as the major ubiquinone). However, we refuse to assign the MB strains on the basis of their ubiquinone type to the subgenus *Gluconoacetobacter*, which was proposed by Yamada and Kondô (39) and comprised the species *A. liquefaciens* and *Acetobacter xylinum* (38); there are too many distinguishing features between these two taxa.

The MB strains exhibit a combination of properties which have not been described previously for *Acetobacter* species. They represent a uniform group of acetic acid bacteria which should be accepted as a separate species, for which we propose the name *Acetobacter methanolicus* (meth. a.nó. li. cus. Lat. n. *methanolum* methanol; L. adj. *methanolicus* methanolic, using methanol as a sole carbon source). The type strain of the new species, strain MB58, has been deposited with the Culture Collection of the Institute of Microbiology and Experimental Therapy (IMET) of the Academy of Science of the German Democratic Republic, Jena, as strain IMET 10945^T. A description of the species is given below.

***Acetobacter methanolicus* sp. nov.** Cells ellipsoidal to rod shaped (0.6 to 0.8 by 1.0 to 1.8 μm) and occur singly, in pairs, or rarely in short chains. Gram-negative. Usually motile and peritrichously flagellated. Resting stages are not formed.

Colonies on methanol-containing mineral agar are circular with entire margins, slightly convex, and weakly glistening, have a moist and smeary consistency, and are white to pale yellowish (rarely pink).

Temperature optimum, 30 to 32°C.

Growth occurs at pH values of <6.5; optimum pH, 4.0 to 4.5.

Strictly aerobic. Metabolism respiratory, never fermentative. Catalase positive. Nitrate is not reduced to nitrite. Gelatin is not liquefied. H_2S and indole are not formed. Methanol is incorporated via the fructose biphosphate variant of the hexulose monophosphate pathway. Enzymes of the tricarboxylic acid cycle are present. The Voges-Proskauer reaction with lactate is negative.

Good growth at pH 4.0 to 5.4 on methanol, glucose, gluconate, glycerol, caproic acid, 2,3-butanediol, and succinic acid. Weak to very weak growth on ethanol, acetate, fumaric acid, propionate, pyruvate, and glyoxylate. No growth on lactate. The utilization of fructose and mannitol by strains differs.

Ammonium and nitrates are utilized as sole sources of nitrogen in the presence of growth factors when methanol, glucose, and other carbon compounds are used as carbon sources.

Pantothenic acid or yeast extract is required as a growth factor.

Ethanol is oxidized to acetic acid, but oxidation to CO_2 and H_2O occurs only at initial ethanol concentrations of <0.5%; lactate is oxidized very weakly.

Acid is formed from glucose, galactose, xylose, L-arabinose, mannose, melibiose, glycerol, methanol, ethanol, *n*-propanol, *n*-butanol, isobutanol, and amylalcohol. On mannitol different strains produce different reactions.

Ketogenesis from glycerol (weak), but not from sorbitol and mannitol; 2-, 5-, or 2,5-ketogluconic acid, γ-pyrones, and brown pigments are not formed.

Ubiquinone type, Q_{10}.

The description of the type strain is the same as that given above for the species, except for the characteristics shown in Table 4. The color of the colonies is white to pale yellow. The guanine-plus-cytosine content of the DNA is 62.3 mol% (thermal denaturation method).

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