Physiological, Biochemical and Morphological Characteristics of Mesquite Wood-digesting Bacteria

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Ten species of bacteria, belonging to the genera *Pseudomonas*, *Bacillus*, *Flavobacterium* and *Brevibacterium*, were isolated from soil or decaying mesquite wood by enrichment culture techniques using mesquite wood as the carbon and energy source. Eight of these species hydrolysed carboxymethylcellulose but none consistently hydrolysed filter paper strips in peptone/NaCl broth. In general, they were proteolytic, non-fermentative in Hugh & Leifson's medium, utilized Krebs' cycle intermediates as sources of carbon and possessed carboxymethylcellulase.

INTRODUCTION

Mesquite brush (genus *Prosopis*) infests over 50 million acres in the State of Texas. The density of the brush cover, coupled with its very large thorns, greatly reduces the value of the land for grazing cattle. Research in this laboratory demonstrated the suitability of mesquite wood as a carbon and energy source for the growth of selected bacteria to produce a feed supplement or, potentially, a complete cattle feed (Thayer *et al.*, 1975; Chang & Thayer, 1975; Fu & Thayer, 1975; Thayer, 1976*a*). The bacteria used in these studies were isolated from termites (Thayer, 1976*b*) and by enrichment culture techniques from soil and decaying wood. This paper describes the physiological and morphological properties of the strains of bacteria isolated by enrichment culture techniques from soil or decaying mesquite wood.

METHODS

Several species of bacteria were isolated by enrichment culture techniques. The basal medium, pH 6.45, contained (g l⁻¹): NaCl, 6.0; (NH₄)₂SO₄, 1.0; KH₂PO₄, 1.0; K₂HPO₄, 1.0; MgSO₄·7H₂O, 0.10; CaCl₂, 0.01; Difco yeast extract, 0.5; and mesquite wood (harvested while dormant in March and milled into sawdust), 10.0. Inocula (10.0 g) for enrichment cultures, obtained from decaying mesquite wood or the soil below it, were added to 100 ml medium in 500 ml baffled Erlenmeyer flasks. Cultures were incubated at 30 °C and agitated at 250 rev. min⁻¹ for 3 days. Each third day thereafter, 10 ml was removed to inoculate a sterile flask of mesquite medium for 10 successive transfers. From several such enrichment cultures, 65 isolates were obtained which grew well in the mineral salts medium containing mesquite wood as the major carbon source. Ten isolates were selected for further study because of their superior growth (as measured by viable cell number, bacterial proteins and substrate hydrolysis) on mesquite wood.

The morphological, cultural, biochemical and physiological characteristics were determined in duplicate by procedures described previously (Thayer, 1976*b*). Uninoculated and/or negative controls were included where appropriate. Flagellar morphology was confirmed by examining specimens, negatively stained with 2 % (w/v) phosphotungstic acid, in an Hitachi HS-8-2 electron microscope.

RESULTS

The morphological, biochemical and physiological characters of the 10 strains are given in Tables 1 to 5.

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Table 1. *Morphological and biochemical properties of wood-digesting bacteria*

Character	JM 127	JM 92	JM 106	JM 99A	JM 99B	JM 98A	JM 66C	JM 68B	JM 116	JM 90
Cell morphology										
Gram reaction	—	—	—	+	+	+	+	+	+	+
Endospores	—	—	—	—	—	—	—	+	+	+
Motility	+	—	—	—	—	—	—	—	+	—
Metachromatic granules	—	NT	—	—	—	—	—	—	—	+
Growth										
In TSB+5.0% (w/v) NaCl	+	+	—	+	+	+	+	+	+	+
In TSB+7.0% (w/v) NaCl	—	—	—	+	+	+	—	+	+	—
In TSB+10.0% (w/v) NaCl†	—	—	—	—	—	—	—	—	+	—
In presence of 0.001% lysozyme‡	+	+	+	—	—	—	—	+	—	—
At pH 5.7, at 30 °C	±	+	+	—	—	—	—	—	—	+
At 41.5 °C in TSB	+	—	+	+	+	+	—	+	+	+
At 40 °C in TSB	+	—	+	+	+	+	—	+	+	+
On α -cellulose agar	+	NT	+	+	NT	+	+	+	NT	NT
On CM-cellulose agar	±	NT	+	±	NT	±	±	+	NT	NT
On microcrystalline cellulose agar	±	+	+	+	—	+	+	+	±	+
On microcrystalline cellulose agar + yeast extract	+	±	+	+	—	+	+	+	+	+
Anaerobic in Brewer's thioglycollate	+	+	+	—	+	+	+	+	+	+
On BBL Pseudose agar	+	—	—	—	—	—	—	—	—	—
Biochemical tests										
Gelatin hydrolysis	—	—	+	+	+	+	+	+	—	+
Casein hydrolysis	—	—	—	+	+	+	+	+	—	+
Urea hydrolysis	—	—	+	—	—	—	—	—	—	—
Tryptophan hydrolysis	—	—	—	±	—	±	—	—	—	+
Lipid hydrolysis	—	+	+	—	—	—	+	—	—	—
Lecithin hydrolysis	—	—	—	—	—	—	—	+	—	—
Amylose hydrolysis	*	—	*	+	*	+	*	*	+	*
Pectin hydrolysis	—	—	—	—	—	—	—	—	+	—
CM-cellulose gel hydrolysis	—	+	+	+	+	+	+	—	+	+
DNA hydrolysis	—	—	NT	—	—	—	+	—	+	—
Filter paper breakage	—	—	—	—	—	—	—	—	1/6	1/6
Methyl red test	—	—	—	—	—	—	—	+	—	—
Acetoin production	—	—	+	—	—	—	—	+	—	—
Catalase	+	+	+	+	+	+	+	+	—	+
Cytochrome oxidase	+	+	+	—	—	+	+	+	+	—
Peroxidase	+	+	+	—	—	+	+	+	+	—
L-Arginine decarboxylase	+	—	—	—	—	—	—	—	—	—
Nitrate reduction	—	—	—	—	—	—	—	+	+	+
Tyrosine decomposition	+	+	—	±	±	+	+	±	—	±
Dihydroxyacetone production	—	—	—	—	—	—	—	—	+	—
L-Arginine dihydrolase	+	±	±	±	±	—	—	—	—	+

+, Positive; —, negative; ±, doubtful; NT, not tested; 1/6, one of six tests positive.

* Weak positive reaction below growth.

† No culture grew in the presence of 15% (w/v) NaCl.

‡ Gordon, Haynes & Pang (1973).

None of the organisms grew in KCN broth. All gave negative results in tests for phenylalanine deaminase, L-lysine decarboxylase, L-ornithine decarboxylase, and pigment production in the presence of 0.01% tyrosine. The *Bacillus* species JM68B, JM116, and JM90 failed to grow at either pH 7 or pH 6 at 60 °C; the remaining strains were not tested for this property.

Strain JM127 was identified as a species of the genus *Pseudomonas*. The differentiating characteristics of this organism were: unicellular, non-photosynthetic, non-sporeforming Gram-negative rod-shaped cells, 0.50 to 0.51 × 0.85 to 2.15 μ m, occurring singly, motile with monotrichous or multitrichous polar flagella, chemo-organotrophs, oxidative by the Hugh & Leifson (1953) method. Neither vitamins nor amino acids were required for growth. It may be related to *Pseudomonas caryophylli* based on the description of Doudoroff &

Table 2. *Acid formation from carbohydrates*

Carbohydrate	JM 127	JM 92	JM 106	JM 99A	JM 99B	JM 98A	JM 66C	JM 68B	JM 116	JM 90
Bromothymol blue broth base										
Monosaccharides										
D-Fructose	—	—	±	+	+	+	—	+	+	+
D-Galactose	+	+	—	—	—	—	—	—	—	—
D-Glucose	+	+	+	+	+	+	—	+	+	+
D-Mannose	+	+	+	+	—	+	—	—	+	—
Rhamnose	—	—	—	+	+	+	—	—	—	—
Disaccharides										
β-D-Cellobiose	—	—	+	—	+	+	+	—	+	—
Lactose	—	—	—	—	—	—	—	—	—	+
Maltose	—	—	+	+	+	+	+	+	—	+
Melibiose	—	—	—	+	—	+	—	—	—	—
Sucrose	—	—	—	+	—	+	—	+	+	+
Trehalose	—	—	—	—	—	—	—	+	+	—
Polysaccharides										
Inulin	—	—	—	—	—	—	—	—	+	—
Melezitose	—	—	—	+	+	+	—	—	—	—
Alcohols										
Glycerol	—	—	—	—	—	—	—	+	+	—
Mannitol	—	—	—	+	+	+	—	—	—	—
Glucosides										
Aesculin	—	—	—	+	+	—	—	—	+	—
Salicin	—	—	+	+	+	+	—	—	+	+
Hugh & Leifson (1953) media										
Glucose, aerobic	+	—	—	—	—	—	—	—	+	—
Glucose, anaerobic	NG	—	NG	—	—	NG	NG	+	+	—
Gordon media										
Glucose slants	+	+	+	+	+	+	+	+	+	+
Glucose stabs	+	+	+	—	—	+	—	+	—	—

—, Acid not produced; +, acid produced; ±, slightly acid; NG, no growth.

None of the organisms formed acid from L-arabinose, D-xylose, α-amylase, raffinose, adonitol, dulcitol, inositol or sorbitol in bromothymol blue broth base. Strains JM127, JM106, JM98A and JM66C did not produce acid aerobically and did not grow anaerobically in phenol red broth or Hugh & Leifson media containing sucrose or lactose. Strain JM92 produced acid from glucose in phenol red broth, but not in any of the Hugh & Leifson media. Strain JM106 did not produce acid aerobically from glucose in phenol red broth and failed to grow anaerobically in the same medium. Strain JM90 produced acid from xylose on Gordon's medium; strains JM68B and JM116 did not.

Palleroni (1974). At variance with this identification is its inability to reduce nitrate. After 5 days incubation on Difco Tryptic Soy Agar (TSA), colonies of JM127 were circular, convex, cream to tan, slightly translucent, 3 mm diam., had an entire margin, and did not produce soluble pigment. Abundant growth occurred on BBL Pseudosel agar (King, Ward & Raney, 1954); after 4 days incubation the colonies were light tan, small (2 mm diam.), convex or umbonate, and did not produce soluble pigment. On Difco Pseudomonas P agar, colonies were round, translucent, light tan, about 3 mm diam., with an entire margin and a convex elevation; no soluble pigment was produced. On Difco Pseudomonas F agar, a water-soluble light-green fluorescent pigment was produced. The colonies were round with one concentric ring, an entire margin and umbonate elevation; they were 2 mm diam., light tan and had a slightly granular appearance. A pellicle was formed on liquid culture media. The most unusual characteristics of strain JM127 were its ability to rapidly degrade mesquite wood and to produce cellulolytic enzymes during growth (Thayer *et al.*, 1975; Thayer, 1976c).

Table 3. *Assimilation of organic acids from a modified Christensen medium (Yamada & Komagata, 1972)*

Acid	JM 127	JM 92	JM 106	JM 99A	JM 99B	JM 98A	JM 66C	JM 68B	JM 116	JM 90
Pyruvic acid	+	+	—	+	+	+	+	+	+	+
Fumaric acid	+	+	+	+	+	+	NT	+	+	+
α -Ketoglutaric acid	+	+	—	+	+	+	NT	+	+	+
Citric acid	+	+	+	+	+	+	—	+	+	+
Propionic acid	+	+	+	+	+	+	—	+	—	±
Butyric acid	+	+	—	+	+	+	NT	+	—	+
Oxalic acid	+	—	+	+	+	+	+	+	+	+
Glutaric acid	+	+	—	—	—	—	NT	—	—	+
Adipic acid	+	+	—	+	+	+	NT	—	—	+
Pimelic acid	—	—	—	—	—	—	NT	—	—	+
Glycollic acid	—	—	—	—	—	—	NT	—	—	—
Hippuric acid	+	+	+	+	+	+	NT	+	+	+
Uric acid	+	—	—	+	+	+	NT	—	—	—

+, pH change from neutral to alkaline; —, no pH change; NT, not tested.

All strains used acetic, lactic, malic, succinic, formic and malonic acids.

Strain JM92 cells were short rods, $0.5 \times 1.0 \mu\text{m}$, or cocci, $0.5 \mu\text{m}$ diam., though some were lancet shaped. They were Gram-negative and non-motile. Colonies at 48 h and 35°C on standard agar media or TSA were round, with an entire margin and convex or drop-like elevation; they were 0.5 to 0.75 mm diam., smooth, glistening, translucent and yellow. Acid but no gas was produced from glucose. Lactose was not fermented. Strain JM92 was similar to *Flavobacterium breve*.

Strain JM106 was a small Gram-negative rod, 0.8 to 1.0×1.2 to $2.0 \mu\text{m}$. Cells occurred singly and had round ends. Colonies on TSA were pale yellow, round, convex, opaque, glistening, smooth and 1 to 3 mm diam. Cultures in BBL Trypticase Soy Broth (TSB) became very turbid after 24 h, but very little growth occurred in nutrient broth. This bacterium appeared to belong to the genus *Flavobacterium* but did not correspond with any species described in *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974).

Strain JM99A cells were Gram-positive rods, 0.6×1 to $2 \mu\text{m}$, though some were curved. They did not have metachromatic granules. Colonies on TSA were light lemon yellow, raised, circular, 1 to 3 mm diam. and glistening. On cellulose agar, colonies were cream and spreading. There was no apparent clearing of the cellulose. Cells were non-motile and tended to be grouped in palisade arrangements. Cellulose strips were not hydrolysed in 0.5 % peptone. Metabolism was oxidative but acid was produced from a number of carbohydrates. The same general description applied to JM98A and JM99B, although there were several differences between the individual strains. These three strains were placed in the genus *Brevibacterium*. The uncertain status of this genus prevented their speciation.

Strain JM66C formed colonies on TSA which were initially colourless, but later turned cream; they were flat with raised centres, smooth, umbonate, translucent, shiny and 1 to 1.5 mm diam. after 24 h. The cells were Gram-variable rods, 0.5×1 to $2.5 \mu\text{m}$, though some were in palisade type groupings. This strain is apparently an unusual *Brevibacterium* species.

Strain JM68B had rod-shaped cells, 0.9 to 1.0×1.5 to $3.5 \mu\text{m}$, with flat to slightly concave ends. Endospores were formed. The sporangia were not swollen. Colonies on TSA were slightly irregular, beaded, erose-edged, off-white, opaque and dull-surfaced. A ring, turbidity and large amounts of off-white sediment were produced in TSB. Very poor growth occurred on Difco potato dextrose agar. The differentiating characteristics of this organism were: Gram-positive cells, ellipsoidal subterminal endospores that did not distend the

Table 4. Assimilation of compounds as sole carbon source

Compound	JM 127	JM 92	JM 99A	JM 99B	JM 98A	JM 68B	JM 116	JM 90
α -D-Fucose	—	—	—	—	—	—	+	—
Galactose	+	+	—	—	—	—	—	—
D-Glucose	+	+	—	—	—	—	—	—
Fructose	+	—	—	—	—	—	—	—
Sucrose	—	—	—	—	—	—	+	—
Xylose	+	+	—	—	—	—	—	—
Acetic acid	+	+	+	—	—	—	—	—
Adipic acid	—	+	—	—	—	—	—	—
Benzoic acid	—	+	—	—	—	—	—	—
Butyric acid	+	+	—	—	—	—	—	—
Citric acid	+	+	+	+	+	—	+	—
Formic acid	—	—	—	—	—	—	+	—
Fumaric acid	+	+	+	+	+	—	+	—
Hippuric acid	—	+	—	—	—	—	—	—
<i>p</i> -Hydroxybenzoic acid	—	+	+	+	+	—	—	—
DL- β -Hydroxybutyric acid	+	+	—	—	—	—	—	—
2-Keto-D-gluconic acid	+	—	+	+	+	±	—	—
α -Ketoglutaric acid	+	+	—	—	—	—	—	—
Lactic acid	+	+	—	—	—	—	+	—
Malic acid	+	+	—	+	—	—	+	—
Malonic acid	—	+	—	—	—	—	—	—
Nononic acid	+	+	—	—	—	—	—	—
Propionic acid	+	—	—	—	—	—	—	—
Pyruvic acid	+	+	—	—	—	—	—	—
Succinic acid	+	+	+	+	+	—	+	+
Uric acid	+	—	—	+	+	—	—	—
Butan-1-ol	—	+	—	—	—	—	—	—
Ethanol	—	+	—	—	—	—	±	—
Propan-1-ol	+	+	—	—	—	—	—	—
β -Alanine	+	+	—	—	—	+	—	—
DL-Alanine	+	+	+	—	—	+	+	—
γ -Aminobutyric acid	+	+	—	—	—	+	+	—
DL-Arginine	+	—	+	+	+	+	+	+
L-Aspartic acid	+	+	+	+	+	+	+	—
Betaine.HCl	+	—	+	—	—	+	+	+
L-Cysteine	—	—	—	—	—	+	—	—
Glutamic acid	+	+	—	—	+	—	—	—
L-Glutamine	+	+	+	+	—	+	+	—
DL-Histidine	+	+	+	+	+	+	+	—
DL-Isoleucine	±	—	—	—	—	—	—	—
L-Isoleucine	+	—	—	—	—	+	—	—
DL-Leucine	+	—	—	—	—	—	—	—
L-Leucine	+	—	—	—	—	+	—	—
DL-Methionine	+	—	—	—	—	—	—	—
L-Phenylalanine	+	+	—	—	—	+	—	—
L-Proline	+	+	+	+	+	—	+	—
DL-Serine	+	—	—	—	+	+	—	—
L-Serine	+	—	—	—	±	+	—	—
DL-Threonine	—	—	—	—	—	+	—	—
DL-Tryptophan	—	+	—	—	—	—	—	—
L-Tyrosine	+	+	—	—	—	+	±	—

+, Growth occurred on two consecutive transfers on the same medium; ±, very small amount of growth; —, no growth.

None of the organisms grew without an added carbon source. Strains JM106 and JM66C did not grow on any compound tested singly. None of the organisms used glycollic, tartaric or oxalic acids, nor lactose, maltose, cellobiose, α -cellulose, mannitol, glycine, L-valine, L-cystine, DL-norleucine, methanol or propan-2-ol as sole carbon sources.

Strains JM127, JM99A, JM99B and JM98A required neither vitamins nor amino acids for growth though these substances greatly promoted growth. Strains JM92, JM68B, JM116, JM90 and JM66C required amino acids for growth.

Table 5. *Antibiotic sensitivity*

Antibiotic	JM 127	JM 92	JM 106	JM 99A	JM 99B	JM 98A	JM 66C	JM 68B	JM 116	JM 90
Bacitracin (10 u.)	—	±	±	+	+	+	+	±	—	++
Dihydrostreptomycin (100 µg)	++	++	—	++	++	++	++	+	+	++
Erythromycin (50 µg)	—	+	+	++	+	+	++	++	++	++
Erythromycin (150 µg)	—	++	++	++	+	++	++	++	++	++
Furadantin (1.5 mg)	—	—	+	—	—	—	—	++	++	++
Kanamycin (100 µg)	++	++	—	+	+	+	++	+	++	+
Nalidixic acid (100 µg)	—	—	++	±	—	—	—	±	++	—
Neomycin (100 µg)	+	+	+	++	+	±	++	+	+	++
Penicillin G (5 u.)	—	—	—	—	±	+	+	+	++	+
Penicillin G (10 u.)	—	—	—	+	+	+	++	+	++	+
Streptomycin (100 µg)	+	++	—	++	+	+	++	+	+	++
Sulphathiazole (3 mg)	—	—	—	++	++	++	±	±	+	±
Tetracycline (100 µg)	±	—	+	++	++	++	++	++	+	++

—, No zone of inhibition; ±, small zone of inhibition; +, moderate zone of inhibition; ++, large zone of inhibition.

sporangium, catalase positive, produced acid from glucose, produced acetoin but not gas. Strain JM68B was classified as a variety of *Bacillus megaterium*, most likely the subspecies *Bacillus carotarium*.

The differentiating characteristics of strain JM116 were: Gram-positive rod-shaped cells, 0.5 to 0.75 × 2 to 3 µm, ellipsoidal endospores in a central or subterminal position that did not distend the sporangium. Colonies on TSA were irregular, about 3 to 10 mm diam. after 5 days incubation, umbonate, with wrinkled surfaces, lobate-edged, white and opaque. A pellicle and flocculent sediment were produced in TSB. There was abundant, white growth on potato dextrose agar. The organism required one or more amino acids for growth. It hydrolysed carboxymethylcellulose gels very rapidly. Strain JM116 may be related to *Bacillus pulvifaciens*.

Strain JM90 had large (1.0 to 1.5 × 3 µm) Gram-positive rod-shaped cells with cylindrical and subterminal endospores; the cells stained poorly with the Gram stain and appeared foamy. Colonies at 24 h on TSA were 1.5 to 3.0 mm diam., mucoid, shiny, convex and off-white. Seven-day-old colonies on TSA were 1 to 3 mm diam., smooth, raised, entire-edged, opaque, dull and off-white. Strain JM90 was catalase positive and may be related to *B. pulvifaciens*.

DISCUSSION

All the strains described here were isolated by enrichment culture techniques with ground mesquite wood as the source of carbon. These 10 strains were selected for further study on the basis of their ability to grow rapidly on the wood. Thus all were presumed to have some ability to degrade and utilize the components of wood. All strains were isolated from soil and/or decaying wood though none are phytopathogens. None could weaken filter paper sufficiently for it to break during every test. However, all but two strains hydrolysed carboxymethylcellulose gels. Of the strains tested, all except JM99B grew on α-cellulose, carboxymethylcellulose or microcrystalline cellulose agars. Other activities which might be associated with wood degradation such as amylose hydrolysis, pectin hydrolysis and utilization of xylose were not common.

Some cellulolytic activities of these strains have been described previously, and presumptive evidence was found for the degradation of lignin by JM127, JM68B, and JM99A (Thayer *et al.*, 1975). In a later report, Thayer (1976c) questioned the validity of the cellulose strip hydrolysis test and tentatively proposed that strains JM98A, JM99A and JM99B were non-typical Cellulomonas strains. However, Braden & Thayer (1976) found the cell wall antigens from

JM98A and JM99A had a low serological cross-reaction with any of six authentic *Cellulomonas* species and concluded that they were not members of that genus.

The majority of the 10 isolates utilized citrate, fumarate, succinate, alanine, arginine, aspartic acid, glutamine, histidine and proline. Most could assimilate more compounds than could be used as sole sources of carbon. Thus they tended to be proteolytic, oxidative and capable of utilizing Krebs's cycle intermediates as sole sources of carbon.

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REFERENCES

- BRADEN, A. R. & THAYER, D. W. (1976). Serological study of *Cellulomonas*. *International Journal of Systematic Bacteriology* **26**, 123-126.
- BUCHANAN, R. E. & GIBBONS, N. E. (editors) (1974). *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore: Williams & Wilkins.
- CHANG, W. T. H. & THAYER, D. W. (1975). The growth of *Cytophaga* on mesquite. *Developments in Industrial Microbiology* **16**, 456-464.
- DOUDOROFF, M. & PALLERONI, N. J. (1974). Genus I. *Pseudomonas* Migula 1894. In *Bergey's Manual of Determinative Bacteriology*, 8th edn. p. 217. Edited by R. E. Buchanan and N. E. Gibbons. Baltimore: Williams & Wilkins.
- FU, T. T. & THAYER, D. W. (1975). Comparison of batch and semicontinuous cultures for production of protein from mesquite wood by *Brevibacterium* sp. JM98A. *Biotechnology and Bioengineering* **17**, 1749-1760.
- GORDON, R. E., HAYNES, W. C. & PANG, G. H.-N. (1973). *The Genus Bacillus*. Agricultural handbook no. 427. Washington, D.C.: Agricultural Research Services, United States Department of Agriculture.
- HUGH, R. & LEIFSON, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *Journal of Bacteriology* **66**, 24-26.
- KING, E. O., WARD, M. K., & RANEY, D. E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory Clinical Medicine* **44**, 301.
- THAYER, D. W. (1976a). A submerged culture process for production of cattle feed from mesquite wood. *Developments in Industrial Microbiology* **17**, 79-89.
- THAYER, D. W. (1976b). Facultative wood-digesting bacteria from the hind-gut of the termite *Reticulitermes hesperus*. *Journal of General Microbiology* **95**, 287-296.
- THAYER, D. W. (1976c). Cellulolytic and physiological activities of bacteria during production of single-cell protein from wood. In *Proceedings of the American Institute of Chemical Engineers Eighty-First National Meeting* p. 44, paper no. 17b.
- THAYER, D. W., YANG, S. P., KEY, A. B., YANG, H. H. & BARKER, J. W. (1975). Production of cattle feed by the growth of bacteria on mesquite wood. *Developments in Industrial Microbiology* **16**, 465-474.
- YAMADA, K. & KOMAGATA, K. (1972). Taxonomic studies on coryneform bacteria, IV. Morphological, cultural, biochemical and physiological characteristics. *Journal of General and Applied Microbiology* **18**, 399-416.