Journal of General Microbiology (1976), **95**, 287–296
Printed in Great Britain

Facultative Wood-digesting Bacteria from the Hind-gut of the Termite *Reticulitermes hesperus*

By D. W. THAYER

Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409, *U.S.A.*

(*Received* 10 *November* 1975; *revised* 16 *January* 1976)

SUMMARY

Among the facultative bacteria capable of growth on mesquite wood which were isolated from the asceptically dissected hind-gut of the termite *Reticulitermes hesperus* were two strains of *Bacillus cereus*, one strain each of Arthrobacter, Alcaligenes and Serratia, and a very small Gram-negative fermentative rod. The *B. cereus* strains, the *Serratia marcescens* strain and the *Arthrobacter* sp. grew well on a mineral salts α-cellulose agar. One of the *Bacillus cereus* strains and *Serratia marcescens* hydrolysed gels of carboxymethylcellulose. All isolates grew well with mesquite wood as the carbon source. The *Serratia marcescens* isolate produced prodigiosin but differed from a typed strain both in size and in some physiological characteristics.

INTRODUCTION

Mesquite brush (genus *Prosopis*) cannot be digested by cattle in its native form, but aerobic, submerged cultivation of bacteria on the mesquite wood increases the digestible protein and exposes the wood structure to the digestive processes of the ruminant (Thayer *et al.*, 1975). Many sources of bacteria were considered for this process, including the hind-gut of the termite. Since wood contains many possible substrates, bacteria capable of utilizing mesquite as their carbon and energy source would not necessarily be cellulolytic.

Several species of bacteria which grew rapidly on media containing mesquite wood as the carbon source were isolated from the hind-gut of the termite. One of these species, designated *Serratia marcescens* RW3, was used in the laboratory for production of single-cell protein (Thayer *et al.*, 1975). This strain and others isolated from the hind-gut possess cellulolytic enzymes (Thayer *et al.*, 1975). In view of their possible commercial application a more detailed study of these bacteria seemed justified. This paper describes the physiological and morphological properties of strains of bacteria isolated from the hind-gut of the termite.

METHODS

Reticulitermes hesperus was isolated from infested wood in Oklahoma and Texas. Hind-guts were dissected aseptically from worker termites which had been washed in Wescodyne antiseptic (West Chemical Products, New York, New York 11101, U.S.A.; U.S. pat. no. 2710277) and then in sterile water to suppress surface bacteria. Samples (0·01 and 0·04 ml) of the hind-gut contents were mixed with physiological saline (0·35 or 1·95 ml, respectively), and spread plates were prepared on BBL Trypticase soy agar (TSA). Cultures were incubated at 30 °C until maximum colony development had occurred. Each colony type was

restreaked several times in sequence on TSA to obtain pure cultures. They were characterized by the following methods.

Morphological characteristics. Bacterial form, size, pleomorphism and Gram-stain reaction were determined with bacteria grown on TSA, BBL Trypticase soy broth (TSB), or TSA containing 1 % (w/v) glucose. Motility was investigated by hanging-drop flagellation confirmed by staining (Leifson, 1951). Metachromatic granules were observed in bacteria grown on TSA plus glucose for 24 h by the staining method of Skerman (1967), and fuchsin stains were prepared by the technique of Smith, Gordon & Clark (1952).

Cultural characteristics. Growth, surface structure, pigmentation, etc. were observed after 1 to 10 days incubation on TSA, nutrient agar, or TSA plus 1 % (w/v) glucose. Basal media, pH 7·0, contained (g/l distilled water): NaCl, 3·0; KH_2PO_4, 1·0; K_2HPO_4, 1·0; $MgSO_4$, 0·05; $CaCl_2$, 0·05; special Noble Agar (Difco), 20·0; and either carboxymethylcellulose, 10·0; or α-cellulose, 10·0; or Cellex MN (BioRad Laboratories, Richmond, California 94804, U.S.A.), microcrystalline cellulose, 0·5; or Cellex MN, 0·5, and yeast extract, 0·5. The media were sterilized by autoclaving for 15 min at 121 °C and poured into Petri plates.

Biochemical characteristics. Fermentative abilities were determined in phenol red broth base (Difco) or bromothymol blue broth base (Difco) containing filter-sterilized carbohydrate (5·0 g l^{-1}). The type of glucose, lactose and sucrose cleavage was determined by the Hugh & Leifson (1953) method. Glucose and xylose fermentation were rechecked on slants by the technique of Gibson & Gordon (1974). Cultures were incubated at 35 °C for 21 days.

Organic acid assimilation was tested by using a modified Christensen's medium (Yamada & Komagata, 1972). Cultures were incubated at 35 °C for 21 days. A change in the pH from neutral to alkaline was considered a positive result. Uninoculated controls were incubated under identical conditions. Organic acids or carbohydrate assimilation, as sole carbon source, was assayed with the Stanier, Palleroni & Doudoroff (1966) medium. Assimilation was indicated by growth and a change in the pH of the medium, provided that the culture retained viability upon transfer to the same medium at 35 °C.

Extracellular deoxyribonuclease (DNAase) was detected with DNAase test medium (Difco) after abundant growth had occurred at 35 °C. Urease was detected with urea broth (Difco). Cellulolytic activity was tested with a filter paper strip–peptone medium (Skerman, 1967). Casein hydrolysis was determined after growing the culture on a medium containing 20 ml skim milk, autoclaved for 10 min at 114 °C, per ml TSA. Pectinase was detected using sodium polypectate with TSA as a basal medium (Skerman, 1967). Peroxidase was determined by the Anderson (1930) procedure. Dihydroxyacetone production from glycerol was determined according to Shimwell, Carr & Rhodes (1960). Hydrolysis of carboxymethylcellulose (CM-cellulose) was detected by the liquefaction of a gel containing (g/l distilled water): sodium carboxymethylcellulose (type 7H; Hercules Inc., Wilmington, Delaware, U.S.A.), 20·0; NaCl, 3·0; $(NH_4)_2SO_4$, 8·0; KH_2PO_4, 1·0; K_2HPO_4, 1·0; $MgSO_4$, 0·05; $CaCl_2$, 0·05; Casamino acids (Difco), 0·50. The pH was adjusted to 6·5 and 10 ml portions of the medium were placed in 16 × 150 mm test-tubes. Duplicate tubes were inoculated by stabbing, and incubated for 21 days. In the presence of carboxymethylcellulase, the gel was hydrolysed to a water-like viscosity. Uninoculated controls were always included.

Other tests for phospholipase and lipase activity, nitrate reduction, indole production, phenylalanine deaminase, L-lysine decarboxylase, L-arginine decarboxylase, L-ornithine decarboxylase, methyl red and Voges–Proskauer reactions, and α-amylase were tested according to Holding & Collee (1971), and tyrosinase by the disappearance of crystals of L-tyrosine (0·5 %) in nutrient agar (Gordon, Haynes & Pang, 1973).

Prodigiosin was extracted and purified according to the alkaline extraction procedure of

Hubbard & Rimington (1950) using *Serratia marcescens* Nima as a control (Williams *et al.*, 1971). Water-soluble pigment(s) were extracted from 5-day-old surface-grown *Serratia marcescens* Nima and *S. marcescens* RW3 by suspending bacteria in distilled water and then centrifuging for 30 min at 10000 *g*. Extracts were filtered through a 0·2 μm membrane filter which removed most of the pigment from the former extract but not from the latter. Alcohol-soluble pigment(s) were extracted from intact bacteria in a similar way using absolute ethanol, and clarifying the suspensions by centrifuging at 10000 *g*. Spectra were obtained with a Perkin Elmer Model 402 double-beam recording spectrophotometer with a path length of 1 cm.

Physiological characteristics. The effect of NaCl on growth in TSB containing NaCl at 0·5, 5·0, 7·0, 10·0, and 15·0 % (w/v) was determined at 30 °C. Nutritional requirements were determined using media based on those of Knight & Proom (1950). Ammonia basal medium consisted of (g l^{-1}): KH_2PO_4, 1·5; $(NH_4)_2HPO_4$, 7·0; $MgSO_4.7H_2O$, 0·5; $CaCl_2.2H_2O$, 0·3; $MnSO_4.4H_2O$, 0·04; $FeSO_4.7H_2O$, 0·0025; glucose, 10·0. Basal salts–casein hydrolysate medium consisted of basal salts medium plus 4·0 g vitamin-free Casamino acids (Difco). Basal salts–yeast extract medium consisted of basal salts medium plus 1·0 g of yeast extract (Difco). Basal salts–casein hydrolysate–yeast extract medium included both yeast extract and Casamino acids in the concentrations given above. Other media were medium 7AA and medium 14AA of Knight & Proom (1950). All media were sterilized by filtration. Bacteria were grown in TSB at 30 °C, centrifuged and washed twice in sterile physiological saline. Two portions of each medium (5 ml) were inoculated with 0·05 ml washed bacteria suspended in their original volume of physiological saline. If growth occurred, a loopful was used to inoculate another tube of the same medium. The test was considered positive only when the culture remained viable upon transfer to the same medium. Sensitivity to antibiotics and other agents was determined using the sensitivity disc (Difco) technique. The filter-paper discs were placed on inoculated TSA plates and the zones of inhibition measured after 48 h incubation at 35 °C.

All cultures were incubated at 35 °C unless otherwise indicated. All tests were performed in duplicate.

RESULTS

The bacterial flora, which grew well on ordinary culture media, of the hind-gut of the termite was consistent in both relative numbers and types. The same species of bacteria were isolated over a period of several months from dissected hind-guts of many termites obtained from both the Texas and Oklahoma colonies. Seven different bacteria were isolated and designated RW1 to RW7. Organism RW4 was not isolated consistently from hind-guts and so was not studied further. The biochemical and physiological characteristics of the others are given in Tables 1 to 5. Preliminary characterization of these strains indicated that there were two strains of Bacillus, one strain each of Arthrobacter, Alcaligenes and Serratia, and a very small fermentative Gram-negative rod designated RW2. The Serratia species was present in the fewest numbers, usually less than 3 % of the total colonies. Strain RW2 usually represented about 14 % of the total colonies, and RW1, RW5, RW6 and RW7 were about equally represented.

Strain RW2 grew very slowly on TSA or other culture media and formed colonies which remained minute after several days' incubation. The organism was a motile Gram-negative coccus, 0·31 by 0·31 μm, usually in pairs, which rarely separated and might therefore be a bipolar staining cell, 0·31 by 0·62 μm. The organism was penicillin resistant. It could not be classified using *Bergey's Manual of Determinative Bacteriology* (1974); though two genera

Table. 1. *Morphological and biochemical characteristics of bacteria isolated from termites*

Character	RW1	RW2	RW3	RW5	RW6	RW7	Nima†
Cell morphology							
Gram reaction	+	−	−	−	+	+	−
Endospores	+	−	−	−	−	+	−
Motility	+	+	+	+	+	+	+
Metachromatic granules	+	NT	NT	NT	NT	+	NT
Growth							
KCN broth	−	NT	−	NT	−	−	−
TSB+5·5 % (w/v) NaCl	+	NT	+	NT	+	+	+
TSB+7·0 % (w/v) NaCl	−	+	−	+	+	−	−
TSB+10·0 % (w/v) NaCl	−	NT	−	NT	−	−	−
In presence of lysozyme	+	NT	+	NT	−	+	+
At pH 7, at 60 °C	−	NT	−	NT	−	−	−
At pH 6, at 60 °C	−	NT	−	NT	−	−	−
At pH 5·7, at 30 °C	−	NT	+	NT	−	−	+
On α-cellulose agar	+	NT	+	NT	+	+	NT
On CM-cellulose agar	±	NT	±	NT	+	+	NT
On microcrystalline cellulose agar	±	NT	±	NT	NT	NT	NT
On microcrystalline cellulose agar+yeast extract	+	NT	+	NT	NT	NT	NT
Anaerobic in Brewer's thioglycollate	+	NT	+	NT	NT	+	+
Biochemical tests							
Gelatin hydrolysis	+	−	+	−	−	+	+
Casein hydrolysis	+	−	+	−	−	+	+
Urea hydrolysis	−	−	−	−	−	−	−
Tryptophan hydrolysis	−	+	+	−	−	−	+
Lipid hydrolysis	−	NT	+	NT	−	−	+
Lecithin hydrolysis	+	NT	+	NT	−	+	+
Amylose hydrolysis	+	−	*	−	*	*	*
Pectin hydrolysis	−	NT	−	NT	−	−	−
CM-cellulose gel hydrolysis	+	NT	+	NT	−	−	NT
DNA hydrolysis	−	NT	+	NT	+	+	+
Methyl red test	+	+	+	−	−	−	+
Acetoin production	+	−	+	−	+	+	+
Catalase	+	+	+	−	+	+	+
Cytochrome oxidase	+	NT	−	NT	+	+	−
Peroxidase	+	NT	−	NT	+	+	−
Phenylalanine deaminase	−	NT	−	NT	−	−	−
L-Lysine decarboxylase	−	NT	+	NT	−	−	+
L-Arginine decarboxylase	+	NT	−	NT	−	−	−
L-Ornithine decarboxylase	−	NT	+	NT	−	−	+
Nitrate reduction	+	−	+	−	−	+	+
Tyrosine decomposition	−	NT	+	NT	−	−	+
Pigment production in presence of 0·01 % tyrosine	−	NT	−	NT	−	−	−
Dihydroxyacetone production	−	NT	+	NT	NG	−	+

+, Positive; −, negative; ±, doubtful. NG, no growth occurred; NT, not tested.
* Weak positive reaction below growth.
† *Serratia marcescens* Nima included as reference strain.

appeared somewhat similar, *Escherichia* and *Wolbachia*. Because of its very slow growth, RW2 was not of commercial interest and was not included in further studies.

Strain RW5, identified as a probable Alcaligenes species, was a Gram-negative rod, 0·64 by 1·28 μm. Its metabolism was oxidative (Table 2), and it utilized citrate.

Strains RW1 and RW7 were both *Bacillus cereus* but differed in a number of characters.

Table 2. *Acid formation from carbohydrates*

RW6 did not form acid from carbohydrates.

Carbohydrate	RW1	RW2	RW3	RW5	RW7	Nima†
Bromothymol blue or phenol red broth base*						
L-Arabinose	−	+	−	−	−	−
D-Xylose	−	+	−	+	−	−
Rhamnose	−	+	−	−	−	−
D-Glucose	+	+o	+	+	+	+
D-Fructose	+	+	+	−	+	+
D-Mannose	−	+	+	−	−	+
D-Galactose	−	+	+	+	−	−
Sucrose	+	+	+	−	+	+
Lactose	−	+	−	−	−	−
Maltose	+	+	+	−	+	+
Trehalose	+	NT	+	NT	+	+
β-D-Cellobiose	+	NT	−	NT	−	−
Raffinose	−	+	−	−	−	−
Melezitose	−	NT	−	NT	−	−
Dextrin	−	NT	−	NT	−	−
Starch	+	NT	−	NT	−	−
Inulin	−	+	−	−	−	−
Glycerol	+	−	+	−	+	+
Adonitol	−	NT	−	NT	−	−
Mannitol	−	NT	+	NT	−	+
Dulcitol	−	NT	−	NT	−	−
Sorbitol	−	NT	−	NT	−	+
Inositol	−	NT	−	NT	−	+
Aesculin	+	NT	+	NT	−	+
Salicin	+	NT	+	NT	−	+
Melibiose	−	NT	−	NT	−	−
Hugh & Leifson media						
Glucose, aerobic	−	+	+	−	−	+
Glucose, anaerobic	−	+	+	−	−	+
Sucrose, aerobic	−	NT	+	NT	−	NT
Sucrose, anaerobic	−	NT	+	NT	−	NT
Lactose, aerobic	−	NT	−	NT	−	NT
Lactose, anaerobic	−	NT	−	NT	−	NT
Glucose broth, aerobic	±	NT	+	NT	+	NT
Glucose broth, anaerobic	±	NT	+	NT	+	NT
Gordon glucose slants	+	NT	+	NT	+	+
Gordon glucose stabs	+	NT	+	NT	+	+
Gordon xylose slants	−	NT	NT	NT	−	NT

−, Acid not produced; +, acid produced; +o, acid and gas produced; NT, not tested.

* Acid formation by strains RW1, RW3, RW6, RW7, and Nima was determined in bromothymol blue broth base. Acid formation by strains RW2 and RW5 was determined in phenol red broth base.

† *Serratia marcescens* Nima included as reference strain.

Strain RW1 was a large, Gram-positive, endospore-forming, facultatively anaerobic rod, being 0·86 by 2·26 μm with round ends and not swollen by the cylindrical subterminal endospores. Cells grown on nutrient agar containing 1 % glucose showed unstained areas when lightly stained or when stained with basic fuchsin. Metachromatic granules were observed at each end of the cell. Those bacteria which were peritrichously flagellated were highly motile in hanging drops. Colonies on TSA after 5 days' incubation at 30 °C were 4 to 5 mm diam., rough-surfaced, lobate-edged, flat, opaque, and off-white in colour. It grew well on mineral salts medium containing 1 % (w/v) α-cellulose and could be transferred on the same medium, but grew poorly on the same basal salts medium containing 1 %

Table 3. *Assimilation of organic acids*

All strains used formic, acetic, pyruvic, lactic, and succinic acid; none used adipic, glycollic, or uric acid.

Acid	RW1	RW3	RW6	RW7	Nima*
Malic acid	+	+	−	+	+
Fumaric acid	+	+	±	+	+
α-Ketoglutaric acid	+	+	−	+	+
Citric acid	−	+	+	+	+
Propionic acid	−	+	−	+	+
Oxalic acid	+	+	+	+	NT
Malonic acid	+	+	−	+	+
Glutaric acid	−	NT	−	−	NT
Pimelic acid	−	+	+	−	+
Hippuric acid	+	+	NT	+	+

NT, Not tested.
* *Serratia marcescens* Nima included as reference strain.

Table 4. *Assimilation of compounds as sole carbon source*

None of the organisms used propionic, butyric, glycollic, hippuric, uric, tartaric, adipic, or oxalic acids, nor xylose, lactose, cellobiose, α-amylose, or α-cellulose as sole carbon sources. Strains RW6 and RW7 could not assimilate any of the compounds listed as sole carbon sources.

Organic acid	RW1	RW3	Nima*	Carbohydrate	RW1	RW3	Nima*
Acetic acid	−	+	−	Glucose	−	+	+
Pyruvic acid	−	+	+	Fructose	−	+	−
Lactic acid	−	+	+	Galactose	−	+	+
Malic acid	+	+	+	Sucrose	−	+	+
Succinic acid	−	+	+	Maltose	−	+	+
Fumaric acid	+	+	+	Mannitol	−	+	+
Citric acid	−	+	+				
Formic acid	−	+	+				
Malonic acid	−	−	+				
α-Ketoglutaric acid	−	−	+				

* *Serratia marcescens* Nima included as reference strain.

(w/v) CM-cellulose. Poor growth occurred on agar containing microcrystalline cellulose in the absense of yeast extract, and clear zones did not develop around the colonies. CM-cellulose gel was rapidly hydrolysed from the surface downwards (Table 1). Growth on nutrient agar slants incubated for 24 h at 30 °C was echinate, white and translucent. The presence of 1 % glucose in the nutrient agar gave dry, rough, creamy colonies. Abundant growth occurred on potato dextrose agar (Difco). Anaerobic growth occurred in motility test agar and in Brewer's thioglycollate medium (Difco). Abundant growth occurred upon transfer to basal salts medium containing either Casamino acids, yeast extract or both. A very active phospholipase was secreted from the culture. A 1 cm wide opaque zone and a small but distinct transparent zone at the edge of the colony developed after 4 days' incubation. Other data are presented in Tables 1 to 5.

Strain RW7, also *Bacillus cereus*, was a large Gram-positive, endospore-forming, facultatively anaerobic rod, with rounded ends, being 1 by 4·0 μm when grown on TSA for 24 h at 30 °C. Cells grown on nutrient agar containing 1 % (w/v) glucose stained unevenly with Gram stain or fuchsin. Metachromatic granules were present. Lipid accumulations were demonstrated by staining with Sudan black B. Endospores were cylindrical, completely

Table 5. *Antibiotic sensitivity*

Antibiotic	RW1	RW3	RW6	RW7
Bacitracin (10 u.)	−	−	++	±
Dihydrostreptomycin (10 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.

filling the sporangium; in a few cases, the sporangium appeared swollen. The cells, when motile, were peritrichous though most cells were immotile. Surface colonies on TSA were irregular, approximately 3 mm diam. after 5 days' growth at 30 °C, umbonate, dull-surfaced, erose-edged, white, opaque, and butyraceous. There was abundant opaque, off-white growth on TSA slants with a raised elevation, rough surface, and erose edge. Abundant flocculent growth occurred in TSB, with a ring formation at the surface. No growth occurred on potato dextrose agar. Cells grew anaerobically in motility test medium and Brewer's thioglycollate medium (Difco). Abundant growth occurred on mineral salts agar containing 1 % (w/v) α-cellulose or CM-cellulose. The phospholipase activity was very similar to that of strain RW1. Nutritional requirements appeared to be identical to those of RW1.

Organism RW3 was a strain of Serratia and as Sakazaki (1974) recognized only one species in this genus, *Serratia marcescens*, RW3 is so identified. For this reason a comparison culture of *Serratia marcescens* Nima (Williams *et al.*, 1971) was included in this study.

Serratia marcescens RW3 cells were typical of the genus, i.e. very small Gram-negative rods 0·51 by 0·79 μm, though cells of *S. marcescens* Nima were twice as large. The colonies were typical of *Serratia*. Pigment, which differed from that of *S. marcescens* Nima, was produced at 37 °C but not at 40 °C. Abundant creamy growth occurred on potato dextrose agar. In motility test agar cultures, abundant growth occurred both aerobically and anaerobically, but pigment was only produced aerobically. The strain was actively motile. Both RW3 and Nima produced phospholipase. Abundant growth was produced by the isolate on medium containing α-cellulose, microcrystalline cellulose or CM-cellulose as the carbon source. CM-cellulose gel was slowly hydrolysed (Table 1). Neither the isolate nor the Nima strain required vitamins or amino acids for growth.

Serratia marcescens RW3 fermented galactose, but *S. marcescens* Nima did not. Sorbitol and inositol were fermented by Nima, but not by RW3 (Table 2). Acetic acid was utilized by RW3 as sole carbon source, but not by Nima. Both malonic and α-ketoglutaric acids were utilized as sole carbon sources by Nima but not by RW3 (Table 4).

Since the colours of the colonies were different, the pigments produced by the strains were analysed. Both strains produced prodigiosin or a prodigiosin-like pigment and diffusible water-soluble pigments. Purified prodigiosins from both strains had identical spectra in

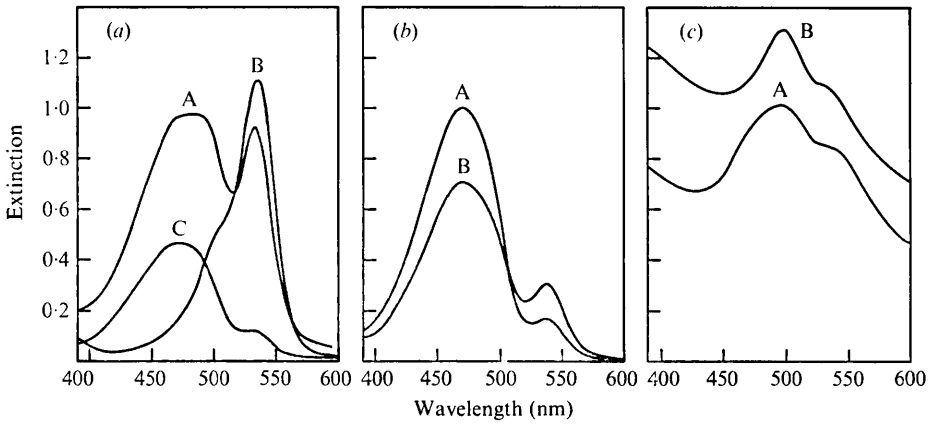

Fig. 1 (*a*). Absorption spectra of purified prodigiosin from *S. marcescens* RW3: A, in 85 % (v/v) ethanol solution; B, in acid (two drops of glacial acetic acid); and C, at pH 10.

(*b*). Absorption spectra of absolute ethanol extracts of *S. marcescens* Nima (A) and *S. marcescens* RW3 (B).

(*c*). Absorption spectra of aqueous suspensions of intact cells of *S. marcescens* Nima (A) and *S. marcescens* RW3 (B).

alkaline, neutral or acidic solutions (Fig. 1 *a*). The alcohol-soluble pigment(s) from intact cells were similar but not identical; that from *S. marcescens* RW3 had a higher extinction at 536 nm but a lower extinction at 468 nm than that of *S. marcescens* Nima (Fig. 1 *b*). The absorption spectra of water-soluble pigments from both strains were identical but more pigment was extracted from strain Nima. Aqueous suspensions of RW3 or Nima cells both had absorption maxima at 490 nm and shoulders at 530 nm but had a different minimum in the 430 to 450 nm region (Fig. 1 *c*). Water-soluble pigments had absorption spectra identical to that of the cells. Further purification of the pigments is necessary to establish if they are identical.

Organism RW6 was typical of the genus *Arthrobacter*. The data were insufficient to allow species identification. Cells underwent a typical change from cocci to rods. Cocci were 0·75 to 1·0 μm diam. in cultures incubated for 2 days or longer; the size gradually increased during further incubation. When these cocci were transferred to fresh medium, they elongated at one or more positions to form rods or Y-shaped cells. The rods were actively motile and 0·5 by 2·5 $\pm$ 2·0 μm. The cocci were usually found in pairs. Growth was slow in TSB and other culture media. Colonies on TSA were circular, 2 mm diam., convex, entire-edged, golden and shiny after 5 days' incubation at 30 °C. Growth on TSA slants was filiform, flat, yellow and glistening. In TSB, the broth was slightly turbid with a definite sediment. Growth did not occur on potato dextrose agar. Abundant growth occurred on mineral salts agar containing 1 % (w/v) α-cellulose or CM-cellulose as the carbon source. The nutritional requirements of this strain were complex since growth did not occur in the mineral salts medium containing both Casamino acids and yeast extract but did occur in the control tube of TSB.

DISCUSSION

Both strains of *Bacillus cereus* grew well on α-cellulose agar and though strain RW1 grew well on CM-cellulose agar it grew poorly on microcrystalline cellulose agar unless

yeast extract was added. The presence of a CM-cellulase was demonstrated in RWI but not in RW7 by the hydrolysis of a CM-cellulose gel. Growth on the α-cellulose agar does not necessarily demonstrate the presence of enzymes capable of solubilizing α-cellulose, as growth may have been due to impurities in the medium, though the α-cellulose used had a stated purity of 99·5 %. Growth of RWI on α-cellulose agar but not on the CM-cellulose agar is further confused by the requirement of RWI for either amino acids or yeast extract. This requirement is the most likely reason for the lack of growth of RWI on α-cellulose as the sole carbon source.

Though both RWI and RW7 were identified as *Bacillus cereus* using standard keys (Gibson & Gordon, 1974; Wolf & Barker, 1968), they were not identical; biochemical differences included DNA hydrolysis by RW7 but not RWI, possession of L-arginine decarboxylase by RWI which fermented cellobiose and starch, and assimilated malic or fumaric acid as a sole carbon source.

Serratia marcescens RW3 was unusual in producing abundant growth on cellulosic media ranging from mesquite wood (Thayer *et al.*, 1975) to CM-cellulose. It was highly fermentative but did not ferment cellobiose. Both RW3 and Nima strains assimilated most of the organic acids investigated and utilized several as their sole carbon source. Evidence was presented in a previous paper for the presence of cellulase active against microcrystalline cellulose and presumptive evidence for the hydrolysis of lignin (Thayer *et al.*, 1975).

Arthrobacter sp. RW6 grew well on α-cellulose and may be significant for this reason. Because its growth rate was slow, it probably would not provide protein for the host termite but it might provide enzymes which would interact with those from other organisms.

This study has not clarified the role of the bacteria associated with the flagellate protozoans of termite hind-guts. The methods which were chosen for cultivation allowed the growth of only the free living and relatively non-fastidious bacterial species. Hypermastigote flagellates were observed in the gut contents along with their associated spirochaetes. Cleveland & Grimstone (1964) described the fine structure of the flagellate *Mixotricha paradoxa* and its associated micro-organisms.

Relatively few studies have been made of the bacteria associated with the termite and only a few obtained evidence for any cellulolytic activity. Dickman (1931) found many different cellulose digesting organisms in the nest walls but not in the intestinal contents of *Reticulitermes flavipes*. Beckwith & Rose (1929) reported cellulose digestion by bacteria isolated from *Reticulitermes hesperus* and several other species. These workers incubated their cultures aerobically as was done in this study. Hungate (1946) isolated an anaerobic cellulose-decomposing actinomycete, *Micromonospora propionici*, from the crushed alimentary tract of a worker termite, *Amitermes minimus*, but as it grew extremely slowly its importance to the digestion of cellulose by the termite was discounted. Schmidt (1956) described bacterial vacuoles of protozoa isolated from the hind-gut of *Reticulitermes lucifregus* and attributed the digestion of wood to the bacteria. Kovoor (1968) found pockets containing bacteria which she designated as a fermentation chamber in the hind-gut of *Microcerotermes edantatus*. The species isolated from the hind-gut were unable to degrade filter paper strips. All, however, grew rapidly on media containing ground mesquite wood as the sole carbon source and produced cellulases. These strains may interact with the cellobiase-producing bacteria in the hind-gut to achieve rapid wood digestion.

The author wishes to thank R. Winegar who performed the initial isolations from the hind-gut, and C. Lindner, J. V. Perez, and K. R. Schulze for their technical assistance. The author acknowledges the culture of *Serratia marcescens* Nima provided by R. P. Williams.

This investigation was supported by funds from the Brush Control and Range Improvement Association, the Dodge Jones Foundation, and the State of Texas.

REFERENCES

ANDERSON, L. R. (1930). Peroxidase in relation to bacterial growth with special reference to the influenza bacillus. *Journal of Bacteriology* **20**, 371–379.

BECKWITH, T. D. & ROSE, E. J. (1929). Cellulose digestion by organisms from the termite gut. *Proceedings of the Society for Experimental Biology and Medicine* **27**, 4–6.

BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY, 8th edn (1974). Edited by R. E. Buchanan and N. E. Gibbons. Baltimore: Williams and Wilkins.

CLEVELAND, L. R. & GRIMSTONE, A. V. (1964). The fine structure of the flagellate *Mixotricha paradoxa* and its associated microorganisms. *Proceedings of the Royal Society* B **159**, 668–686.

DICKMAN, A. (1931). Studies on the intestinal flora of termites with reference to their ability to digest cellulose. *Biological Bulletin. Marine Biological Laboratory, Woods Hole, Mass.* **61**, 85–92.

GIBSON, T. & GORDON, R. E. (1974). Genus I. Bacillus Cohn 1872. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, p. 529. Edited by R. E. Buchanan and N. E. Gibbons. Baltimore: Williams and Wilkins.

GORDON, R. E., HAYNES, W. C. & PANG, C. H.-N. (1973). *The Genus Bacillus.* Agricultural handbook no. 427. Washington, D.C.: Agricultural Research Services, United States Department of Agriculture.

HOLDING, A. J. & COLLEE, J. G. (1971). Routine biochemical tests. In *Methods in Microbiology*, vol. 6A, pp. 2–32. Edited by J. R. Norris and D. W. Ribbons. London: Academic.

HUBBARD, R. & RIMINGTON, C. (1950). The biosynthesis of prodigiosin, the tripyrrylmethene pigment from *Bacillus prodigiosus* (*Serratia marcescens*). *Biochemical Journal* **46**, 220–225.

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.

HUNGATE, R. E. (1946). Studies on cellulose fermentation. II. An anaerobic cellulose decomposing actinomycete, *Micromonospora propionici* n.sp. *Journal of Bacteriology* **51**, 51–56.

KNIGHT, B. C. J. G. & PROOM, H. (1950). A comparative survey of the nutrition and physiology of mesophilic species in the genus *Bacillus*. *Journal of General Microbiology* **4**, 508–538.

KOVOOR, J. (1968). L'intestin d'un termite superieur (*Microcerotermes edantatus* Was., Amitermitinae). Histophysiologie et flore bactérienne symbiotique. *Bulletin Biologique de la France et de la Belgique* **102**, 46–84.

LEIFSON, E. (1951). Staining, shape and arrangement of bacterial flagella. *Journal of Bacteriology* **62**, 377–389.

SAKAZAKI, R. (1974). Genus IX. Serratia Bizio 1823. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, p. 326. Edited by R. E. Buchanan and N. E. Gibbons. Baltimore: Williams and Wilkins.

SCHMIDT, H. (1956). Studien an darmbewohnenden Flagellaten der Termiten. II. Mitteilung. Holzteilchen als Zelleinschlüsse und Nahrung bei *Trichonympha* and Joenia. *Zeitschrift für Parasitenkunde* **17**, 269–275.

SHIMWELL, J. L., CARR, J. G. & RHODES, M. C. (1960). Differentiation of *Acetomonas* and *Pseudomonas*. *Journal of General Micribiology* **23**, 283–286.

SKERMAN, V. B. D. (1967). *A Guide to the Identification of the Genera of Bacteria*, 2nd edn. Baltimore: Williams and Wilkins.

SMITH, N. R., GORDON, R. E. & CLARK, F. E. (1952). *Aerobic Sporeforming Bacteria.* Agricultural monograph no. 16. Washington, D.C.: United States Department of Agriculture.

STANIER, R. Y., PALLERONI, N. J. & DOUDOROFF, M. (1966). The aerobic pseudomonads: a taxonomic study. *Journal of General Microbiology* **43**, 159–271.

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.

WILLIAMS, R. P., GOTT, C. L., QADRI, S. M. H. & SCOTT, R. H. (1971). Influence of temperature of incubation and type of growth medium upon pigmentation in *Serratia marcescens*. *Journal of Bacteriology* **106**, 438–443.

WOLF, J. & BARKER, A. N. (1968). The genus *Bacillus*: aids to the identification of its species. In *Identification Methods for Microbiologists*, part B. Edited by B. M. Gibbs and D. A. Shapton. London: Academic.

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.