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Transfer of *Corynebacterium pyogenes* (Glage) Eberson to the Genus *Actinomyces* as *Actinomyces pyogenes* (Glage) comb. nov†

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The physiology, metabolism, nutrition, and biochemical characteristics of *Corynebacterium pyogenes* (Glage) Eberson were studied in detail to determine the taxonomic status of this organism. *C. pyogenes* is a gram-positive, nonmotile, nonsporulating, short, rod-shaped bacterium which produces acid but not gas from a variety of carbohydrates. A number of amino acids were tested and did not appear to serve as sources of energy for growth. *C. pyogenes* is urease and catalase negative, does not reduce nitrates, and does not produce indole. Wide zones of β-hemolysis on blood agar, acid coagulation of litmus milk, and digestion of the clot are characteristic. Growth is comparable under aerobic and strictly anaerobic conditions. Metabolism is strictly fermentative. Glucose is fermented in CO_2-containing media to succinate, acetate, formate, and lactate; no propionic acid is produced. In identical media without CO_2, lactate is the major product, and only small amounts of acetate, succinate, and formate are produced. Hemin is stimulatory or required for growth. CO_2/HCO_3^- and inositol seem to be obligatory growth factors. Certain peptides appear to relieve the requirement for inositol. All strains require riboflavin and nicotinic acid, and most require adenine and uracil for optimal growth. Characteristic cell wall sugar components are rhamnose and glucose, and the major diamino acid of peptidoglycan is lysine. Cells contain a type *b* cytochrome. Based on these data, we propose that *C. pyogenes* be transferred to the genus *Actinomyces* as *Actinomyces pyogenes* (Glage) comb. nov.

Corynebacterium pyogenes (Glage) Eberson (34) is frequently isolated from a variety of pyogenic disease conditions in cattle, sheep, goats, pigs, and other domestic animals (5, 34) and from humans (1, 8, 29, 41). It has long been recognized that *C. pyogenes* bears little similarity to *Corynebacterium diphtheriae* (the type species) and related animal-pathogenic corynebacteria, such as *Corynebacterium pseudotuberculosis* and *Corynebacterium kutscheri*, and that *C. pyogenes* should be excluded from the genus *Corynebacterium* (2, 10, 14, 19, 20, 23, 34). However, the exact taxonomic status of *C. pyogenes* has remained an enigma due to a lack of sufficient information about this organism. The objective of this investigation was to study the morphology, physiology, metabolism, nutrition, and biochemical characteristics of *C. pyogenes* to determine its taxonomic status. Based on the results of this study, we propose that *C. pyogenes* be transferred to the genus *Actinomyces* (38) as *Actinomyces pyogenes* (Glage) comb. nov.

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MATERIALS AND METHODS

Bacterial strains. Since no type strain was designated for *C. pyogenes* at the time that this work was initiated in 1977, strain Glage C-100 (= NCTC 5224 = ATCC 19411), which is the parent strain of the recently designated type strain (37) of *C. pyogenes*, was obtained from C. S. Cummins, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, and was used in this study. *C. pyogenes* strains NCDO 1903, NCDO 1906, NCDO 1909, NCDO 1910, and NCDO 1911 were from the National Collection of Dairy Organisms, Reading, England, and were sent to us by D. J. Jayne Williams. The source of strain MSU 5 (= ATCC 33157) has been described previously (31). Strains Carter BT-34-49a, BT-337-76, and BT-400-76 were obtained from G. R. Carter of our department. Unless otherwise mentioned, most of the results were obtained with the three well-characterized and widely studied strains C-100, MSU 5, and

† Article 10230 from the Michigan Agriculture Experiment Station.

TABLE 1. Acid production from various carbohydrates and biochemical characteristics of *C pyogenes*

Test	Results of:[a]		
	This study (strain C-100)[b]	Roberts[c]	Barksdale et al.[d]
Acid production from:			
Adonitol	a	17[c]	ND
Amygdalin	–	0	ND
Arabinose	–	37	0
Cellobiose	a	50	ND
Dextrin	a	76	100
Erythritol	a	20	ND
Esculin	–	ND	ND
Fructose	a	83	100
Galactose	a	53	100
Glucose	a	100	100
Glycerol	–	47	0
Glycogen	a	77	ND
Hippurate	–	ND	ND
Inositol	a	21	62
Inulin	–	0	ND
Maltose	a	61	100
Lactose	a	79	100
Mannitol	–	10	0
Mannose	a	43	ND
Melzitose	a	ND	ND
Melibiose	–	ND	ND
Raffinose	–	0	0
Rhamnose	–	ND	0
Ribose	a	ND	ND
Salicin	–	0	ND
Sorbitol	a	6	0
Starch	a	83	19
Sucrose	–	44	29
Trehalose	a	59	0
Xylose	a	70	24
Gelatin hydrolysis	+	ND	24
Milk	A, C, D	A, C, D	A, C, D
Indole	–	0	ND
Nitrate	–	ND	ND
Catalase	–	0	ND
Hemolysis	+	100	100
Urease	–	–	–

[a] Abbreviations: a, acid but no gas; w, weak acid; +, positive test; –, negative test; ND, not determined; A,C,D, acid, clot, and digestion of the clot.

[b] All other strains were identical to strain C-100 in their biochemical characteristics, except that strain NCDO 1909 fermented mannitol and sucrose. Alanine, arginine, aspartate, glycine, and threonine were not fermented by any of the strains.

[c] The numbers are the percentages of positive strains of the 100 strains studied by Roberts (33).

[d] The numbers are the percentages of positive strains of the 21 strains studied by Barksdale et al. (3).

NCDO 1909. Of these, strains C-100 and MSU 5 were identical in all characteristics tested; NCDO 1909 was very similar to the other two strains except for some relatively minor differences, as noted below. All 10 strains were included in some critical experiments, such as those used to determine metabolic products and key biochemical characteristics.

Maintenance of cultures. Cultures were maintained on plates of tryptose agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% sheep blood, as previously described (30).

Media. Serum-free medium (SFM), which was used in most of the experiments, contained (per 100 ml) 0.5 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 0.2 g of yeast extract (Difco), 0.4 g of glucose, 7.5 ml of mineral solutions 1 and 2 of Caldwell and Bryant (7), 0.0002 g of hemin, 1.0 ml of a volatile fatty acid solution (1 ml each of isobutyric, DL-2-methyl butyric, and isovaleric acids and 1.6 ml of acetic acid in 100 ml of water), 2 ml of 1 M potassium phosphate buffer (pH 7.0), 0.05 g of cysteine hydrochloride, and 0.4 g of $NaHCO_3$. All of the ingredients except cysteine and $NaHCO_3$ were mixed in distilled water, the pH was adjusted to 6.5 with 2.5 N NaOH, and the preparation was dispensed into foam-plugged tubes (18 by 150 mm; 9.5 ml/tube) and sterilized by autoclaving at 121°C for 15 min. The cysteine hydrochloride and $NaHCO_3$ solutions were autoclaved separately and were added aseptically to the sterile medium immediately before inoculation. SFM agar medium contained SFM and 1.5 g of agar (Difco) per 100 ml of medium. SFM agar plates were stored and incubated under CO_2 to prevent aklalinization of the medium due to CO_2 loss.

Anaerobic SFM, which in addition contained 0.0001% (wt/vol) resazurin (Eh indicator), was prepared by using Hungate anaerobic techniques, as modified by Holdeman et al. (22), and was dispensed under 100% CO_2 into sterile tubes (18 by 150 mm; 10 ml/tube) closed with butyl rubber stoppers.

The chemically defined medium (CDM) used for determining the inositol, vitamin, purine and pyrimidine, and peptide requirements for *C. pyogenes* growth was identical in composition to the medium previously described (30), except that inositol was replaced by 0.4 g of charcoal-treated Trypticase (17) per ml, unless otherwise mentioned.

Vitamin requirements were detemined by adding one or more filter-sterilized vitamin solutions, as needed, to sterile CDM lacking vitamins.

The purine and pyrimidine requirements for *C. pyogenes* growth were determined by adding one or more sterile solutions of individual purines or pyrimidines or both to sterile CDM lacking purines and pyrimidines.

To study the CO_2/HCO_3^- requirements of *C. pyogenes*, $NaHCO_3^-$ was deleted from anaerobic SFM, and dissolved CO_2 was rigorously excluded from the medium as previously described by Dehority (15), except that the initial gas stripping was done at pH 5.5. This experiment (see Table 2) was conducted in a Bio-Flo fermentor (model C30; New Brunswick Scientific Co., New Brunswick, N.J.) containing 550 ml of medium. The gas flow rate was 600 ml/min. The fermentor was sparged with 100% CO_2, 100% N_2, or 90% N_2–10% CO_2, as indicated below.

Fractionation of Trypticase. Charcoal-treated Trypticase was fractionated on a Sephadex G-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) column (1.5 by 30 cm). KPO_4 buffer (50 mM, pH 7.0) was used as the eluant at a flow rate of 60 ml/h. A 5-ml portion of a 20% (wt/vol) Trypticase solution was applied to the

TABLE 2. Effect of CO_2/HCO_3^- on the growth of *C. pyogenes*[a]

Gas phase	HCO_3^- added (g/100 ml)	A_{600}[b]
CO_2	0.4	1.60 (26)
N_2	0.006	0.32 (72)
N_2	0.0	0.03 (72)
90% N_2–10% CO_2	0.0	1.35 (30)

[a] *C. pyogenes* was grown in SFM containing varying amounts of added $NaHCO_3$ and was incubated under different gas phases.

[b] Growth was estimated by measuring A_{600}. The values in parentheses are the numbers of hours of incubation required for maximum absorbance.

column, and after the void volume had passed, 5-ml fractions of effluent were collected. The fractions were autoclaved for 10 min before they were added to CDM lacking Trypticase.

The amino nitrogen contents of the above-mentioned fractions were determined by the method of Rosen (35). A volume of each fraction was hydrolyzed with an equal volume of 12 N HCl for 11 h at 121°C. The average peptide size for each fraction was calculated by dividing the value for α-amino nitrogen from the hydrolyzed fraction by the corresponding value for the unhydrolyzed sample.

Growth measurements. Growth was estimated by measuring the absorbance at 600 nm (A_{600}) with a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). The mean absorance values for triplicate tubes are reported below. Other experiments (data not shown) showed good correlations among A_{600} values, cell numbers, and dry weights of cells of strain MSU 5 (A_{600} of 1.0 = $1.83 \times 10^9 \pm 0.08 \times 10^9$ cells per ml = 0.45 mg [dry weight]).

Preparation of inocula. Except for the nutritional experiments, cells grown in SFM at 37°C (A_{600}, 0.8) served as the inocula (0.05 ml/10 ml of medium). For

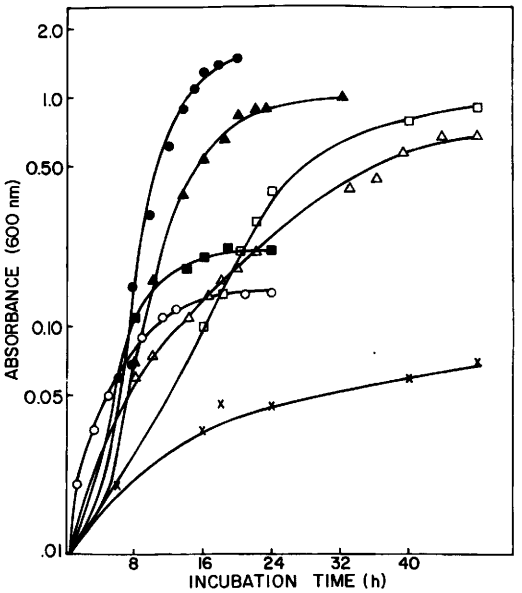

FIG. 1. Effects of various deletions from SFM on the growth of *C. pyogenes* strain MSU 5. Symbols: ●, no deletion; ▲, minus Trypticase; ■, minus yeast extract; ○, minus glucose; △, minus $NaHCO_3$; □, minus hemin; ×, minus hemin and $NaHCO_3$.

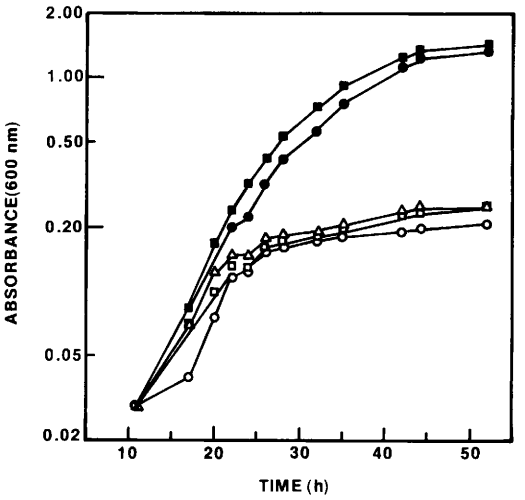

FIG. 2. Purine and pyrimidine requirements for growth of *C. pyogenes* strain MSU 5. Various bases were added to CDM lacking purines and pyrimidines. Symbols: ■, complete complement of purines and pyrimidines; ●, adenine and uracil; △, adenine; □, uracil; ○, no addition.

Preparation of inocula. Except for the nutritional experiments, cells grown in SFM at 37°C (A_{600}, 0.8) served as the inocula (0.05 ml/10 ml of medium). For

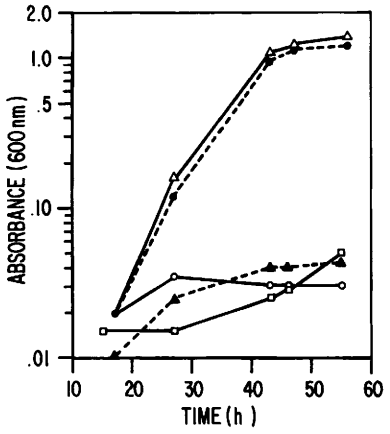

FIG. 3. Inositol requirement for growth. The basal medium used was CDM lacking Trypticase. In different experiments 1 mg of inositol per ml, 1 mg of lipoic acid per ml, or 0.5 mg of Tween 80 per ml was added. When Trypticase was added, it was at the same concentration as in CDM. Symbols: □, no addition; △, plus Trypticase; ●, plus inositol; ○, plus Tween 80; ▲, plus lipoic acid.

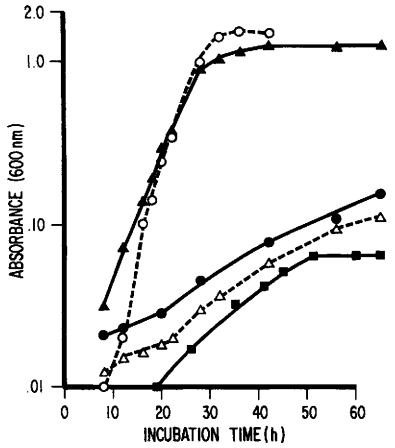

FIG. 4. Effects of deletions of various nitrogen sources from CDM on the growth of *C. pyogenes* strain MSU 5. Nitrogen sources were deleted either singly or in various combinations. Symbols: ■, minus Trypticase; ●, minus Trypticase and cysteine; △, minus Trypticase and amino acids; ▲, minus amino acids; ○, complete CDM.

nutritional experiments (see Fig. 1 through 5 and Tables 2 and 3), cells for inocula were grown at 37°C in CDM (A_{600}, 0.4). Whenever possible, the organism was serially subcultured in a nutritional medium at least three times to minimize the effects of carry-over of any nutrients present in the original inoculum, and the results obtained after the third serial transfer are reported.

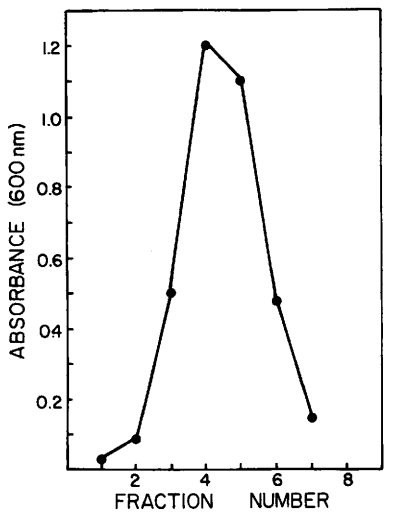

FIG. 5. Maximum growth of *C. pyogenes* strain MSU 5 in response to additions of various Trypticase fractions. Each fraction was added to CDM lacking Trypticase. Charcoal-treated Trypticase (15) was fractionated on a Sephadex G-25 column as described in the text.

Morphology. Cellular morphology was determined by examining wet mounts and Gram-stained smears of cells grown in SFM and on blood agar plates for 18 and 48 h, respectively. Cell dimensions were measured with a calibrated ocular micrometer or were calculated from measurements made from photomicrographs of known magnification.

Ultrastructure was determined by examining electron micrographs of thin sections of cells harvested from a log-phase (12-h) culture of *C. pyogenes* grown in SFM. Cells were fixed by the method of Ryter and Kellenberger (36) and embedded in Epon.

Biochemical characteristics. The biochemical characteristics of *C. pyogenes* were determined under strictly anaerobic conditions by using the procedures described in the *Anaerobe Laboratory Manual* (22) and SFM lacking glucose as the basal medium. Each substrate was added to the basal medium as recommended by Holdeman et al. (22). All biochemical test media were inoculated with 0.05 ml of a 24-h-old culture (A_{600}, 1.5) of *C. pyogenes* grown in SFM and were incubated for 72 h (or longer as needed) at 37°C. All biochemical tests were run in triplicate, and there was good reproducibility of the results among replicates.

Acid metabolic products. An analysis of acid metabolic end products was performed on the supernatants of SFM-grown cultures as previously described (32). A Varian model 2440 chromatograph fitted with a stainless steel column (0.125 in. [3.18 mm] by 6 ft [1.83 m]) packed with 3% SP-1220–1% H_3PO_4 on Chromosorb W-AW (Supelco, Bellefonte, Pa.) was used, with helium as the carrier gas at a flow rate of 25 ml/min. The column temperature was 135°C, the injection temperature was 165°C, the detector current was 225 mA, and the detector temperature was 160°C. The packing used gave good resolution of formic acid from acetic acid.

Cytochrome measurements. The presence of cytochromes was determined by using dithionite-reduced

TABLE 3. Effects of vitamins on the growth of *C. pyogenes*

Addition(s)[a]	Growth of:[b]	
	Strain C-100	Strain MSU 5
None	0.02 (45)	0.17 (46)
Riboflavin	0.11 (37)	0.65 (36)
Nicotinic acid	0.05 (38)	0.10 (50)
Riboflavin + nicotinic acid	0.74 (38)	1.20 (36)
Riboflavin + nicotinic acid + biotin + thiamine	1.1 (38)	
Complete	1.1 (45)	1.5 (36)

[a] None, CDM lacking vitamins; complete, vitamin mixture as in complete CDM. No vitamins or one or more vitamins were added to CDM lacking vitamins at the same concentrations used in CDM.

[b] Growth was measured by A_{600}, as described in the text. The values in parentheses are the numbers of hours of incubation required for maximum absorbance.

minus air-oxidized difference spectra, as previously described (31). Hemes were extracted, the pyridine hemochromes were prepared as previously described (31), and the spectra of both the acid-acetone-soluble and insoluble fractions were determined.

RESULTS

Oxygen relationships. Although *C. pyogenes* has been described as facultatively anaerobic (34), the effect of oxygen on the growth of this organism has never been clearly defined. Therefore, *C. pyogenes* was grown in SFM under the following conditions: (i) strictly anaerobic conditions under 100% CO_2; (ii) aerobically in foam-plugged vessels (incubated without shaking); and (iii) with vigorous continuous aeration by sparging with 95% air–5% CO_2. Growth was essentially identical (A_{600}, 1.5 ± 0.2) under all three conditions, and more than 90% of the glucose carbon utilized was recovered as organic acids. These results indicated that *C. pyogenes* obtains its energy for growth by fermentative metabolism.

Biochemical characteristics. A number of investigators have described the biochemical characteristics of *C. pyogenes* under aerobic growth conditions (3, 32, 33, 39). Since the results of our study indicated that *C. pyogenes* is an anaerobe from a metabolic standpoint, the biochemical characteristics of *C. pyogenes* (Table 1) were determined under strictly anaerobic conditions so that the results obtained would be comparable to those obtained with anaerobes (22, 38). Results obtained under aerobic conditions by Roberts (33) and Barksdale et al. (3) are also presented in Table 1 for comparative purposes. All strains of *C. pyogenes* were very similar in producing acid but not gas from a variety of carbohydrates. However, some strain variations in the fermentation of mannitol and sucrose were observed. Similar results were obtained by Roberts. About 10% of the strains studied by Roberts were positive for mannitol, and about 44% were positive for sucrose fermentation. All of the strains included in this study and in that of Roberts were negative for catalase and positive for gelatin liquefaction. Acid coagulation of milk followed by complete digestion of the clot was characteristic of all strains included in this study.

Acid metabolic products. It has been well established that metabolic products represent an important and stable basis upon which to differentiate fermentative bacteria (32). Furthermore, metabolic products are known to be similar among the strains within each species and are reproducible from culture to culture within a strain (26). Hence, in addition to the 10 strains included in this study, 9 additional strains of *C. pyogenes* were also included in this experiment.

Reddy et al. (31, 32) showed that in Trypticase broth and in brain heart infusion broth with no added HCO_3^-, the major product (>1 meq/100 ml) of glucose fermentation by *C. pyogenes* is lactate and that acetate is a minor product (<1 meq/100 ml). All strains examined in this study produced major amounts of lactate and minor amounts of acetate and succinate in brain heart infusion broth and in SFM lacking HCO_3^-. In SFM, succinate, acetate, and formate were the major products, and variable amounts of lactate were produced. The following acid fermentation products (in millimoles per 100 millimoles of glucose metabolized) were produced in SFM, in SFM lacking HCO_3^-, and in SFM lacking hemin by strain MSU 5: in SFM, formate (61), acetate (66), succinate (85), lactate (24), and pyruvate (3); in SFM lacking HCO_3^-, formate (0), acetate (4), succinate (22), lactate (141), and pyruvate (26); and in SFM lacking hemin, formate (5), acetate (46), succinate (47), lactate (88), and pyruvate (6.9). The CO_2/HCO_3^- fixed in SFM was stoichiometric with the succinate formed. Results were similar with strains C-100 and NCDO 1909.

Cytochrome *b* production. The presence or absence of different types of cytochromes is of some importance in bacterial taxonomy (27). Reddy et al. (31) previously reported the presence of a typical type *b* cytochrome, but not of type *a*, type *c*, or other cytochromes, in cell extracts of strain MSU 5, as revealed by absorption maxima at 560, 530, and 428 nm in a dithionite-reduced minus air-oxidized difference spectrum. The difference spectrum of the pyridine hemochrome derivative of the acid-acetone-extractable heme from *C. pyogenes* showed absorption maxima at 556, 521, and 418 nm, which are characteristic of protoheme, the prosthetic group of cytochrome *b*. The presence of cytochrome *b* has also been demonstrated in strain ATCC 19411 and in a freshly isolated MSU SS 7-74, *C. pyogenes* strain, (M. Kao and C. A. Reddy, unpublished data). Preliminary results showed that the cytochrome *b* in *C. pyogenes* mediates the reduction of fumarate to succinate, with reduced nicotinamide adenine dinucleotide as the electron donor (Cornell and Reddy, unpublished data).

Nutrition. Some nutritional features of *C. pyogenes* MSU 5 are shown in Fig. 1. The results with strains C-100 and NCDO 1909 were identical. *C. pyogenes* appears to require a fermentable carbohydrate for growth since minimal growth was obtained in SFM lacking glucose. Deletion of yeast extract from SFM decreased growth by ~80%. Growth was negligible after simultaneous deletion of hemin and HCO_3^-. Single deletions of hemin and HCO_3^- resulted in ~35 and 50% decreases in growth, respectively (Fig. 1). These results suggested that hemin and

HCO_3^- are stimulatory for growth and that each of these compounds could partially substitute for the other. The doubling time in SFM was 1.6 h, compared with 8.4 h in SFM lacking HCO_3^- and 4.1 h in SFM lacking hemin. Rigorous exclusion of HCO_3^- from the medium by using the procedures of Dehority (15) showed that HCO_3^- is an obligate requirement for the growth of *C. pyogenes* (Table 2). There was no detectable growth in anerobic SFM lacking HCO_3^- when a 100% N_2 gas phase was used. Growth was near normal in the latter medium when a 90% N_2–10% CO_2 gas mixture was substituted for 100% N_2. Appreciable growth was observed in modified SFM containing only 0.006% (wt/vol) HCO_3^-.

Deletion of vitamins from CDM resulted in negligible growth (Table 3). Single additions of nicotinic acid and riboflavin produced little improvement in growth. Combined addition of riboflavin and nicotinic acid gave only 53% of the growth observed in complete medium. Strain MSU 5 produced as much growth with riboflavin and nicotinic acid as strain C-100 did with riboflavin, nicotinic acid, biotin, and thiamin. Results with strain NCDO 1909 were identical to those with strain C-100.

Deletion of purines and pyrimidines from CDM resulted in very little growth of strain MSU 5 (Fig. 2). Single additions of adenine and uracil did not appreciably improve growth. Combined addition of adenine and uracil produced growth comparable to that observed in CDM. Very similar results were obtained with strain C-100, but strain NCDO 1909 did not show a requirement for purines or pyrimidines for growth.

Inositol was obligatorily required for growth of *C. pyogenes* strain MSU 5 (Fig. 4). Lipoic acid and Tween 80 were not required for growth. Trypticase appeared to relieve the requirement for inositol. Results were identical with strains C-100 and NCDO 1909.

The effects of deletions of various nitrogen sources from CDM on the growth of *C. pyogenes* strain MSU 5 are shown in Fig. 4. Little growth was observed when Trypticase was deleted from CDM in spite of the fact that the latter medium contained a full complement of amino acids. These results suggested that *C. pyogenes* requires peptides for growth. When charcoal-treated Trypticase was fractionated on a Sephadex G-25 column and each fraction was added individually to tubes containing CDM lacking Trypticase, fraction 4, which had a mean peptide size of 1.5 amino acids, gave the optimal growth response (Fig. 5). Strains C-100 and NCDO 1909 were similar to strain MSU 5 in showing a peptide requirement for growth.

Morphology. After 24 h of incubation on anaerobic blood agar plates, colonies were β-hemolytic, smooth, and circular, with slightly granular surfaces and entire edges. These colonies were convex and translucent to white under reflected light. None of the strains produced a filamentous microcolony under the conditions used. After 48 to 72 h, the colonies were 0.5 to 1.5 mm in diameter, low convex to convex, circular, opaque, white, and soft, with entire edges. Typically, the zones of β-hemolysis were two to three times the diameters of the colonies.

Colonies on anaerobic SFM agar plates were 1.5 to 3 mm in diameter after 24 to 36 h of incubation. Thus, the colonies on SFM developed faster and were typically larger than the colonies on blood agar plates. These colonies were white, raised, translucent, smooth, soft, and circular, with entire to slightly uneven edges.

All strains examined were gram positive, nonmotile, and non-acid fast and showed a certain degree of pleomorphism in different growth media (Fig. 6). Cells from colonies picked from blood agar plates occurred mostly singly and in pairs ("V" and "T" formations) and were 0.2 to 0.5 by 0.6 to 1.9 μm (Fig. 6A). Cells grown in SFM (Fig. 6B) were 0.4 to 0.9 by 1.0 to 2.5 μm and occurred singly, in pairs (V and T formations and some palisades), and often as three-dimensional clusters with apparent branching. Long, multibranched forms were not observed. Brown and Orcutt (4) often observed a mixture of rod-shaped, fusiform, diphtheroid, streptococcal, and some filamentous and branching forms. Some strains tended to "assume more of one form and others another." Streptococcal forms in small clumps and short crooked chains were also observed (4).

Ultrastructure. Transmission electron microscopy of thin sections of *C. pyogenes* showed ultrastructural details typical of gram-positive bacteria. The cells were predominantly rod shaped, although not infrequently rounded forms were also observed. In addition, club-shaped cells (Fig. 7A) and straight to slightly curved rods were seen (Fig. 7B and D). The cytoplasm was granular and packed with ribosomes (Fig. 7B). The nuclear material appeared to be distributed relatively diffusely in the cytoplasm. Division was by septum formation (Fig. 7A and B). Mesosome-like membranous structures were seen in some cells (Fig. 7B and C). The thickness of the cell wall was 29 to 30 nm. The multilayered appearance of the cell wall was rather characteristic (Fig. 7C and D); there was an inner thin darkly staining layer and an outer, thicker, amorphous, lightly staining layer. Thus, the ultrastructure of *C. pyogenes* was very similar to that reported for several *Actinomyces* species (38).

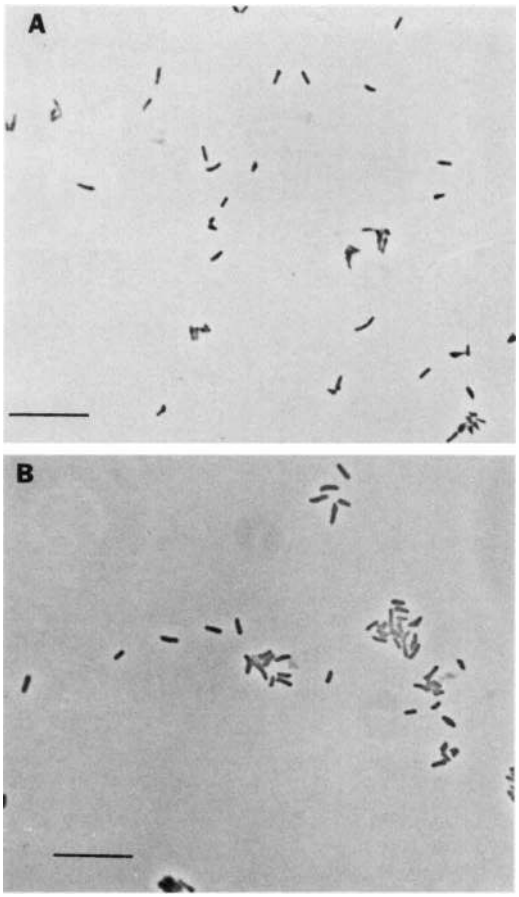

FIG. 6. (A) Photomicrograph of a wet mount preparation of cells of *C. pyogenes* from a 48-h-old colony on a blood agar plate. Bar = 10 μm. Note the rod-shaped to coccobacillary forms occurring singly, in pairs (V and T), in palisades, and in small clumps. Some distinct club-shaped cells are also present. (B) Photomicrograph of a wet mount preparation of *C. pyogenes* cells grown in SFM broth for 18 h. Bar = 10 μm. Note that the cells are generally thicker and longer and have a greater tendency for clumping than the cells grown on blood agar plates.

DISCUSSION

The results of numerous investigations have revealed that *C. pyogenes* is distinctly different from *C. diphtheriae* and related animal-pathogenic corynebacteria in serology (11), in not containing mycolic acids (18), and on the basis of numerical taxonomy (14, 20, 23). The cell walls of *C. pyogenes* differ from those of *C. diphtheriae* and related animal corynebacterial species in containing lysine instead of *meso*-diaminopimelic acid and in containing rhamnose and glucose instead of arabinose and galactose as the major cell wall sugars (11, 13). Experimental results have also shown that *C. pyogenes* differs from *C. diphtheriae* and related corynebacteria in being catalase negative, in liquefying gelatin, in causing acid coagulation and peptonization of litmus milk, and in nutrition. Furthermore, depending on the CO_2/HCO_3^- concentration in the medium, *C. pyogenes* produces lactate or succinate as the major product of glucose fermentation but not propionic acid. In contrast, *C. diphtheriae* and related organisms produce propionate as a major product (32). The results of our study indicate that *C. pyogenes* obtains energy for growth primarily by fermentation. On the other hand, *C. diphtheriae* can grow fermentatively under anaerobic conditions and by aerobic respiration in an oxygen atmosphere (2). Based on these findings, we can safely conclude that *C. pyogenes* has little in common with the type species *C. diphtheriae* and related animal-pathogenic bacteria and should be excluded from the genus *Corynebacterium*, as previously suggested by a number of other investigators (2, 3, 32, 34).

Previous studies have shown that the cell wall composition of *C. pyogenes* is similar to that of *Streptococcus* group G (Table 4) and that these two organisms share a cell wall polysaccharide antigen (3, 11) and ferment similar varieties of carbohydrates (3). Based on these results, Barksdale (2) suggested that *C. pyogenes* taxonomically belongs in the genus *Streptococcus*, specifically in Lancefield group G (16). However, the results of our study, as well as those of other recent investigations (24), show that *C. pyogenes* is different from *Streptococcus* group G. Whereas strains of *Streptococcus* group G, like most other streptococci, are homofermentative lactic acid bacteria, *C. pyogenes* carries out a homolactic acid type of fermentation only in media low in bicarbonate. In contrast, in media containing normal levels of bicarbonate (0.4 g/100 ml), *C. pyogenes* produces major amounts of succinate, acetate, formate, and lactate. This dramatic shift in fermentation depending on the presence of biocarbonate is not known to be exhibited by streptococci (16). Our results show that the amount of CO_2/HCO_3^- fixed is stoichiometric with the succinate formed by *C. pyogenes*, whereas CO_2 fixation to succinate has not been shown for streptococci. The latest edition of *Bergey's Manual of Determinative Bacteriology* considers the absence of cytochromes to be a characteristic of the genus *Streptococcus* (16). In contrast, *C. pyogenes* is known to contain cytochrome *b* (31). The guanine-plus-cytosine content of the deoxyribonucleic acid is ~56 to 58 mol %, which is quite different from the value of 41 mol % reported for *Streptococcus* group G (Table 5). Therefore, it is obvious that *C. pyogenes* is distinctly different from *Streptococcus* group G.

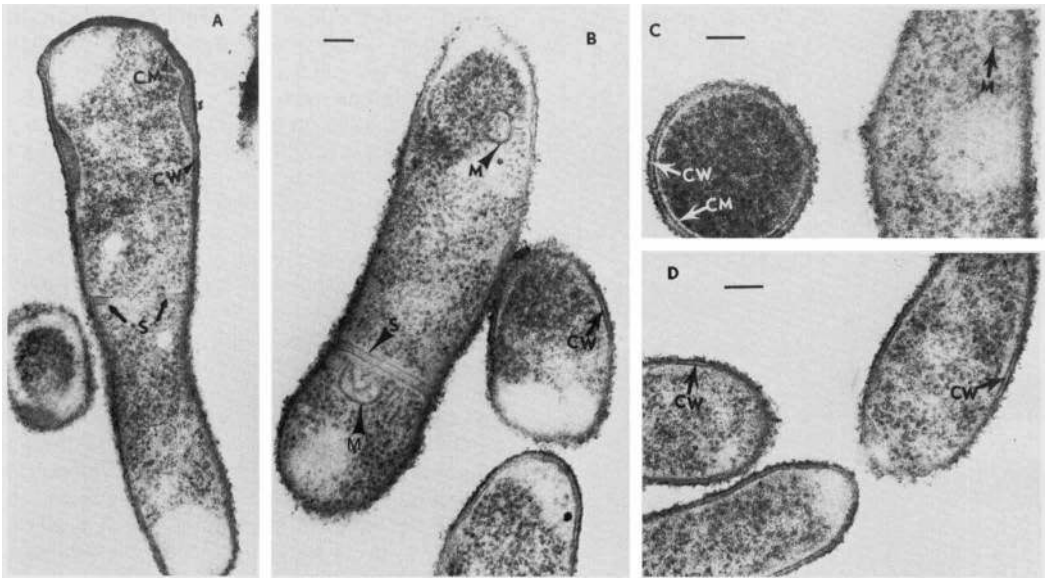

FIG. 7. Thin-section electron micrographs of cells of *C. pyogenes* strain MSU 5 grown in SFM for 12 h. Bar = 0.15 μm. Abbreviations: CM, cell membrane; CW, cell wall; S, septum; M, mesosome-like organelle. (A) Typical club-shaped (coryneform) cells showing clear resolution of the cell wall and cell membrane. Note septal initiation. (B) Cell showing fully formed septum and mesosome-like membranous structures. (C) Transverse section of a cell showing double-track appearance of the cell wall observed in most cells, including some cells in (B) and (D). (D) Angled or V-shaped arrangement of the cells.

The production of formate, acetate, succinate, and lactate as products of glucose fermentation is reported to be an important characteristic of *Actinomyces* species (6, 38, 39). A substantial decrease in succinate production and a corresponding increase in lactate production are observed when *Actinomyces* species are grown in media containing no added HCO_3^- (22, 28). Our results show that *C. pyogenes* is identical to members of the genus *Actinomyces* in the types of fermentation products produced in different media.

The biochemical characteristics (Table 1) of *C. pyogenes* appear to be very similar to those of *Actinomyces bovis* (38, 39), except that *C. pyogenes* is hemolytic and actively proteolytic, as evidenced by its ability to hydrolyze gelatin and casein (milk clot), and produces a soluble toxin. Some strains of *Actinomyces israelii* are known to be proteolytic, as evidenced by zones of hydrolysis on gelatin agar plates (39). *C. pyogenes* strains showed an obligate requirement for HCO_3^- for growth (Table 2), which is in agreement with the finding that HCO_3^- is known to be highly stimulatory or required for the growth of *Actinomyces* species (39). Furthermore, the fact that *C. pyogenes* and *A. israelii* are the only two bacteria that have been shown to require inositol for growth (9; V. B. D. Skerman, Ph.D. thesis, University of Reading, Reading, England, 1966) and the fact that the presence of a type *b* cytochrome has been demonstrated in both *C. pyogenes* and *Actinomyces* species (31, 40) support the concept that these two groups are related to each other. Our results indicate that *C. pyogenes* is an aerotolerant anaerobe. All *Actinomyces* species except *Actinomyces naeslundii* are also aerotolerant anaerobes in that they can grow in air but are not known to utilize oxygen as a terminal electron acceptor in their metabolism. In contrast, *A. israelii* (originally described as *A. naeslundii*) appears to utilize oxygen as a terminal electron

TABLE 4. Cell wall composition of *C. pyogenes* compared with the compositions of *A. bovis*, *C. diphtheriae*, and *Streptococcus* group G cell walls[a]

Organism	Sugars[b]	Amino acids[b]
C. pyogenes	Rha, Glc, ±Man	Ala, Glu, Lys
A. bovis	Rha, 6-D-Tal, Fuc	Ala, Asp, Lys
C. diphtheriae	Ara, Gal, Man	Ala, Glu, *m*-DAP
Streptococcus group G	Rha, Gal	Ala, Glu, Lys

[a] Data from references 3, 11, and 13.

[b] Abbreviations: Rha, rhamnose; Glc, glucose; Man, mannose; 6-D-tal, 6-deoxytalose; Fuc, fucose; Ara, arabinose; Gal, galactose; Ala, alanine; Glu, glutamate; Lys, lysine; Asp, aspartate; *m*-DAP, *meso*-diaminopimelate.

TABLE 5. Comparative guanine-plus-cytosine contents of *C. pyogenes*, certain corynebacteria, *A. bovis*, and *Streptococcus* group G

Organism	Guanine-plus-cytosine content (mol %)	Reference(s)
C. pyogenes	56.3–57.7	24, 34
C. pseudotuberculosis	52.5–53.2	21, 24
C. haemolyticum	48–49	24
C. diphtheriae	52–54.5	21
Streptococcus group G	41.9–41.0	21, 24
A. bovis	54–63	24, 38

acceptor when glucose is metabolized in air (6). The cell wall composition and the guanine-plus-cytosine content of the deoxyribonucleic acid of *C. pyogenes* are very similar to those of *A. bovis*, the type species of *Actinomyces* (Tables 4 and 5). The cellular and colonial morphologies and the ultrastructure of *C. pyogenes* (see above) are also very similar to those of *Actinomyces* species, especially *A. bovis* and *Actinomyces odontolyticus* (39). Serological cross-reaction between *C. pyogenes* and *A. odontolyticus* has also been reported (39).

It is obvious from the discussion above that *C. pyogenes* is similar to members of the genus *Actinomyces* in a number of major characteristics. Therefore, it appears appropriate to transfer *C. pyogenes* to the genus *Actinomyces*, as previously suggested by Slack and Gerencser (39) and Reddy and Kao (32). Furthermore, individual properties of *C. pyogenes* distinguish it from the other currently recognized members of the genus *Actinomyces* (Table 6). Therefore, assignment of separate species status to *C. pyogenes* within the genus *Actinomyces* appears to be justified.

We propose that *C. pyogenes* be transferred to the genus *Actinomyces* and be renamed *Actinomyces pyogenes* (Glage) comb. nov. A comprehensive description of this organism, based on our results and those of other investigators, follows.

Actinomyces pyogenes (Glage) comb. nov. (Gr. n. *pyum* pus; Gr. v. *gennaio* to produce; M. L. adj. *pyogenes* pus-producing). Gram-positive, nonmotile, non-sporeforming coccobacilli and short rods that occur singly, in pairs (V, T, and palisade formations), or as clusters. Short diphtheroid forms with clubs are also seen. Streptococcal forms in small clumps and short crooked chains are occasionally observed (4). Cells vary in shape and size (0.2 to 0.9 by 0.3 to 2.5 μm) in different media. Cells from 24-h-old broth cultures are gram positive, but cells from older cultures may be gram variable. The cell wall ultrastructure is typical of gram-positive bacteria. The cell walls are 29 to 30 nm thick and have a characteristic double-track appearance.

Pinpoint, β-hemolytic colonies occur on sheep blood agar after 24 h of incubation. The zones of hemolysis are typically two to three times the diameter of the colony. After 48 to 72 h, the colonies (0.5 to 1.5 mm) appear convex, circular, opaque, white, and soft, with entire edges. Colonies develop faster and are bigger (1.5 to 3.0 mm) on SFM agar plates.

Good growth occurs under aerobic and strictly anaerobic conditions. Metabolism is strictly fermentative. Acid but not gas is produced from glucose, fructose, galactose, lactose, cellobiose, trehalose, maltose, melzitose, mannose, glycogen, dextrin, xylose, and starch. The fermentation of adonitol, arabinose, erythritol, glycerol, sucrose, mannitol, and sorbitol varies with the strain. No acid from amygdalin, esculin, melibiose, raffinose, rhamnose, or salicin. Alanine, arginine, aspartate, glycine, and threonine are not fermented. Most strains are catalase negative (32), although one strain studied by Cummins (12) has been reported to be catalase positive. Acid clotting and digestion of clots in litmus milk and liquefaction of gelatin are characteristic of all strains. Nitrates are not reduced, and indole is not produced. Optimum temperature, 37°C; temperature range, 20 to 40°C.

Lactic acid is the primary metabolic product in brain heart infusion or tryptose broth with no added HCO_3^-; acetate is a minor product. Glucose is fermented in the presence of CO_2/HCO_3^- and yields succinate, acetate, formate, and lactate as major products. For each 1 mol of CO_2/HCO_3^- fixed, 1 mol of succinate, 1 mol of acetate, and 1 mol of formate are produced. In identical media without added biocarbonate or hemin, lactate is the major product, and smaller amounts of acetate, succinate, and formate are produced.

CO_2/HCO_3^- is required for growth. Hemin is highly stimulatory or required for growth. Peptides are required for growth even in the presence of a complete complement of 20 amino acids and $(NH_4)_2SO_4$. Inositol can replace the peptide requirement for growth. Riboflavin and nicotinic acid are required for optimal growth. Adenine and uracil are required for optimal growth of some strains.

Characteristic cell wall sugar components are rhamnose and glucose, and the major diamino acid of peptidoglycan is lysine (11, 13). No mycolic acids are present (18).

Culture filtrates are fatal to mice and rabbits after intravenous injection. The soluble hemolysin produced is active against human, guinea pig, sheep, horse, and rabbit erythrocytes. Both

TABLE 6. Characteristics differentiating the species of the genus *Actinomyces*[a]

Species	Test results[b]												
	Catalase	Nitrate → nitrite	Milk	Gelatin hydrolysis	Starch hydrolysis	Fermentation of:				Cell wall thickness (nm)	Micro-colonies	Rhamnose in cell wall	Ornithine in cell wall
						Arabinose	Ribose	Xylose	Glycogen				
A. pyogenes	−	−	A, C, D	+	+	−	+	+(−)	+	30	S	+	−
A. bovis	−	d	A, C	−	+	−(+)	−	d	+	31	S	+	−
A. odontolyticus	−	+	A, C	−	−	d	−	d	−	30	S	−[c]	+
A. israelii	−	d	A, C	−	−	d	+	+(−)	−	64	F	−[c]	+
A. naeslundii	−	+	A, C	−	−	−(+)	d	−		45	F	+	+
A. viscosus	+	+	A, C	−	−	−	−	−	−(+)	35	F	+[c]	+

[a] Data from *Bergey's Manual* (38) and this study.
[b] Abbreviations: d, different reactions by different strains; +, positive; −, negative; S, smooth; F, filamentous; A, acid; C, clot; D, digestion of clot. The results in parentheses are rare exceptions.
[c] Cell walls contain galactose.

toxic and hemolytic activities of crude cell extracts are neutralized by antitoxin (25).

The guanine-plus-cytosine content of the deoxyribonucleic acid is 56 to 58 mol %. This organism is frequently isolated from a wide variety of pyogenic disease conditions in many species of domestic animals and in humans (1, 5, 8, 29, 41). Presumably, *C. pyogenes* occurs as a commensal organism on the mucous surfaces of warm-blooded animals (8).

Type strain (37): ATCC 19411 (= NCTC 5224 = C-100).

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ADDENDUM IN PROOF

While this paper was in press, Collins et al. published a paper (M. D. Collins, D. Jones, R. M. Kroppenstedt, and K. H. Schleifer, J. Gen. Microbiol. **128**:335–341, 1982) which strongly supports our proposal for the reclassification of *C. pyogenes* in the genus *Actinomyces*. These investigators, in agreement with the earlier results of Julak et al. (24), showed that tetradecanoic and hexadecanoic acids are the predominant fatty acids in the whole-organism methanolysates of *C. pyogenes*, although large amounts of octadecenoic acid (18:1ω9) are also present. Furthermore, tetrahydrogenated menaquinone with 10 isoprene units was shown to be the predominant quinone in *C. pyogenes*. These results constitute further evidence that *C. pyogenes* does not belong in the genus *Corynebacterium*, that this organism is distinct from *Corynebacterium haemolyticum* and streptococcus group G, and that it is closely related to *A. bovis*. In another paper, G. M. Schofield and K. M. Schaal (J. Gen. Microbiol. **127**:237–259, 1981), showed, on the basis of numerical taxonomy, a close relationship between *C. pyogenes* and *A. bovis*. Thus, there is growing evidence which justifies our proposal for reclassifying *C. pyogenes* as *A. pyogenes*.

REPRINT REQUESTS

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LITERATURE CITED

1. **Ballard, D. O., A. E. Upsher, and D. B. Seeley.** 1947. Infection with *Corynebacterium pyogenes* in man. Am. J. Clin. Pathol. **17**:209–215.
2. **Barksdale, L.** 1970. *Corynebacterium diphtheriae* and its relatives. Bacteriol. Rev. **34**:368–422.
3. **Barksdale, W. L., K. Li, C. S. Cummins, and H. Harris.** 1957. The mutation of *Corynebacterium pyogenes* to *Corynebacterium hemolyticum*. J. Gen. Microbiol. **16**:749–758.
4. **Brown, J. H., and M. L. Orcutt.** 1920. A study of *Bacillus pyogenes*. J. Exp. Med. **32**:219–248.
5. **Bruner, D. W., and J. H. Gillespie.** 1973. Hagan's infectious diseases of domestic animals, 6th ed. Cornell University Press, Ithaca, N.Y.
6. **Buchanan, B. B., and L. Pine.** 1967. Path of glucose

breakdown and cell yields of a facultative anaerobe, *Actinomyces naeslundii*. J. Gen. Microbiol. **46**:225–236.
7. **Caldwell, D. R., and M. P. Bryant.** 1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. Appl. Microbiol. **14**:794–801.
8. **Chlosta, E. M., G. K. Richards, E. Wagner, and J. F. Holland.** 1970. An opportunistic infection with *Corynebacterium pyogenes* producing empyema. Am. J. Clin. Pathol. **53**:167–170.
9. **Christie, A. O., and J. W. Porteus.** 1962. The growth factor requirements of the Wills strain of *Actinomyces israelii* growing in a chemically defined medium. J. Gen. Microbiol. **28**:455–460.
10. **Collins, M. D., T. Pirouz, M. Goodfellow, and D. E. Minnikin.** 1977. Distribution of menaquinones in actinomycetes and corynebacteria. J. Gen. Microbiol. **100**:221–230.
11. **Cummins, C. S.** 1962. Chemical composition and antigenic structure of cell walls of *Corynebacterium, Mycobacterium, Nocardia, Actinomyces* and *Arthrobacter*. J. Gen. Microbiol. **28**:35–50.
12. **Cummins, C. S.** 1971. Catalase activity in *Corynebacterium pyogenes*. Can. J. Microbiol. **17**:1001–1002.
13. **Cummins, C. S., and H. Harris.** 1958. Studies on the cell wall composition and taxonomy of *Actinomycetales* and related groups. J. Gen. Microbiol. **18**:173–179.
14. **Davis, G. H., and K. G. Newton.** 1969. Numerical taxonomy of some named coryneform bacteria. J. Gen. Microbiol. **56**:195–214.
15. **Dehority, B. A.** 1971. Carbon dioxide requirement of various species of rumen bacteria. J. Bacteriol. **105**:70–76.
16. **Deibel, R. H., and H. W. Seeley, Jr.** *Streptococcaceae* fam. nov, p. 490–508. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
17. **Ford, J. E., K. D. Perry, and C. A. E. Briggs.** 1958. Nutrition of lactic acid bacteria isolated from the rumen. J. Gen. Microbiol. **18**:273–284.
18. **Goodfellow, M., M. D. Collins, and D. E. Minnikin.** 1976. Thin-layer chromatographic analysis of mycolic acids and other long-chain components in whole-organism methanolysates of coryneform and related taxa. J. Gen. Microbiol. **96**:351–358.
19. **Gordon, R. E.** 1966. Some strains in search of a genus—*Corynebacterium, Mycobacterium, Nocardia* or what? J. Gen. Microbiol. **43**:329–343.
20. **Harrington, B. J.** 1966. A numerical taxonomic study of some corynebacteria and related organisms. J. Gen. Microbiol. **45**:31–40.
21. **Hill, L. R.** 1966. An index to deoxyribonucleic acid base composition of bacterial species. J. Gen. Microbiol. **44**:419–437.
22. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
23. **Jones, D.** 1975. A numerical taxonomic study of coryneform and related bacteria. J. Gen. Microbiol. **87**:51–96.
24. **Julak, J., M. Mara, B. Potuznikova, and S. Zadrasil.** 1978. Contribution to the taxonomy of haemolytic corynebacteria. Folia Microbiol. **23**:229–235.
25. **Lovell, R.** 1944. Further studies on the toxin of *Corynebacterium pyogenes*. J. Pathol. Bacteriol. **56**:525–529.
26. **Moore, W. E. C.** 1970. Relationship of metabolic products to taxonomy of anaerobic bacteria. Int. J. Syst. Bacteriol. **20**:535–538.
27. **Myer, D. J., and C. W. Jones.** 1973. Distribution of cytochromes in bacteria: relationship to general physiology. Int. J. Syst. Bacteriol. **23**:459–367.
28. **Pine, L., and A. Howell, Jr.** 1956. Comparison of physiological and biochemical characters of *Actinomyces* spp. with those of *Lactobacillus bifidus*. J. Gen. Microbiol. **15**:428–445.
29. **Potocka, F., A. Soucek, and M. Mara.** 1960. New observations on biological properties and toxigenesis of atypical hemolytic corynebacteria isolated from humans. J. Hyg. Epidemiol. Microbiol. Immunol. **4**:307–308.
30. **Reddy, C. A., C. P. Cornell, and A. M. Fraga.** 1980. Chemically defined growth medium for *Corynebacterium pyogenes*. Am. J. Vet. Res. **40**:843–845.
31. **Reddy, C. A., C. P. Cornell, and M. Kao.** 1977. Hemin-dependent growth stimulation and cytochrome synthesis in *Corynebacterium pyogenes*. J. Bacteriol. **130**:965–967.
32. **Reddy, C. A., and M. Kao.** 1978. Value of acid metabolic products in identification of certain corynebacteria. J. Clin. Microbiol. **7**:428–433.
33. **Roberts, R. J.** 1968. Biochemical reactions of *Corynebacterium pyogenes*. J. Pathol. Bacteriol. **95**:127–130.
34. **Rogosa, M., C. S. Cummins, R. A. Lelliott, and R. M. Keddie.** 1974. Coryneform group of bacteria, p. 599–632. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
35. **Rosen, H.** 1957. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys. **67**:10–15.
36. **Ryter, A., and E. Kellenberger.** 1958. Etude au microscope electronique de plasmas contenant de l'acide désoxyribonucléique. I. Les nucléoides des bactéries en croissance active. Z. Naturforsch. Teil B **13**:597–605.
37. **Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.).** 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. **30**:225–420.
38. **Slack, J. M.** 1974. *Actinomycetaceae*, p. 659–681. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
39. **Slack, J. M., and M. A. Gerencser.** 1975. Actinomyces, filamentous bacteria biology and pathogenicity. Burgess Publishing Co., Minneapolis.
40. **Taptykova, S. D., and L. V. Kalakoutskii.** 1973. Low-temperature cytochrome spectra of anaerobic actinomycetes. Int. J. Syst. Bacteriol. **23**:468–471.
41. **Vega, L. E., and T. L. Gavan.** 1970. *Corynebacterium pyogenes*—a pathogen in man. Report of a case. Cleveland Clin. Q. **37**:207–214.