Nocardia coeliaca, *Nocardia autotrophica*, and the Nocardin Strain

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Gray and Thornton's strain (ATCC 17041) of *Mycobacterium coeliacum*, here designated as the type strain of this species (which was later transferred to the genus *Nocardia* as *Nocardia coeliaca*), is a strain of *M. rhodochrous* (tentative generic assignment). The strain producing the antibiotic nocardin and similar strains, however, have been mistakenly labeled *N. coeliaca*. This report presents some of the characteristics of 27 of these mislabeled strains and demonstrates their close relationship to the type strain of *N. autotrophica* (Takamiya and Tubaki) Hirsch. The nocardin-producing strain and others like it are, therefore, assigned to *N. autotrophica*.

In 1928 Gray and Thornton (8) described an isolate from soil and named it *Mycobacterium coeliacum*. Later Jensen (11, 12) studied Gray and Thornton's strain, supplemented its description, and proposed that it typify subgenus B of the genus *Mycobacterium*.

In the 1930's, C. B. van Niel (personal communication) obtained Gray and Thornton's strain of *M. coeliacum* from Jensen and later deposited it in the American Type Culture Collection (ATCC strain 17041). Recently, after examining this strain Jensen (personal communication) confirmed the authenticity of the strain and its history.

When Waksman and Henrici (19) transferred *M. coeliacum* to the genus *Nocardia*, their description of the species was taken from the reports of Gray and Thornton (8) and Jensen (11). Because of its history, its general agreement with Gray and Thornton's original description of *M. coeliacum*, and Jensen's acceptance of it as Gray and Thornton's strain, we designate ATCC strain 17041 as the type strain of *N. coeliaca*.

In 1947 Emmart (5) described nocardin, an antibiotic produced by an air-borne contaminant of a broth culture of *M. tuberculosis*. In Emmart's report, her nocardin-producing strain was described as exhibiting an abundance of aerial hyphae on most media and was named *Nocardia coeliaca* (Gray and Thornton) *nov. comb.* (sic). Emmart's strain and others like it are maintained as *N. coeliaca* in various collections including the National Collection of Industrial Bacteria (NCIB strains 8939 and

9574), the American Type Culture Collection (ATCC strain 13181), and the U. S. Department of Agriculture (NRRL strains B-1365 and B-1807).

We have compared Gray and Thornton's strain (obtained from two sources) with Emmart's nocardin strain and similar ones in our collection. Although chronic "lumpers," we concluded that these strains belong to different species of different genera. Jensen also examined a nocardin strain (NCIB 8939) and concurred with our conclusion (personal communication).

Among the named strains of *Nocardia* species in our collection, only one, a strain of *N. autotrophica* (Takamiya and Tubaki [18]) Hirsch (9), has the same pattern of characteristics as the nocardin-producing strain.

MATERIALS AND METHODS

Organisms. Gray and Thornton's (8) strain of *Mycobacterium coeliacum* was obtained from the ATCC (strain 17041) and from V. B. D. Skerman, University of Queensland (strain 132). The other strains described are listed in Table 1 or by Gordon (6) and Gordon and Horan (7).

Acid fastness. The cultures were grown for 5 days at 28 C on glycerol agar that consisted of: peptone, 5 g; beef extract, 3 g; agar, 15 g; glycerol, 70 ml; soil extract, 150 ml; tap water, 850 ml; adjusted to pH 7.0. The soil extract was prepared by sifting air-dried garden soil through a coarse sieve and autoclaving 400 g with 960 ml of tap water at 121 C for 1 h. After the mixture was cool and settled, the supernatant was carefully decanted, filtered through paper, autoclaved

in 200-ml quantities, and stored at room temperature until cleared by sedimentation.

Smears of the cultures were air dried and immersed in carbol fuchsin (saturated ethanol solution of basic fuchsin, 10 ml; 5% aqueous solution of phenol, 90 ml). The fuchsin was heated and boiled for 5 min. The slides were washed in water, dipped once in acid alcohol (concentrated HCl, 3 ml; 95% ethanol, 97 ml), quickly washed again in water, and counterstained with methylene blue (saturated solution of methylene blue in 95% ethanol, 30 ml; 0.01% aqueous solution of KOH, 100 ml).

Colonial morphology. Cultures grown in glucose broth (5 g each of peptone, beef extract, yeast extract, and glucose, and 1,000 ml of distilled water; pH 7.0) were streaked on plates of tap-water agar (15 g/liter). The plates were incubated at 28 C and examined under a microscope at 5, 10, and 15 days for the appearance of the colonies, the formation of substrate and aerial hyphae, and the presence of spores.

Acid production from carbohydrates. The basal inorganic nitrogen medium contained (grams per liter): $(\text{NH}_4)_2\text{HPO}_4$, 1; KCl, 0.2; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; agar, 15 (1). The pH value of this medium was adjusted to 7.0 before the addition of 15 ml of a 0.04% solution of bromocresol purple. After this agar medium was tubed and sterilized by autoclaving, 0.5 ml of a 10% solution of each carbohydrate (autoclaved separately) was added aseptically to the tubes. Cultures on slants of these carbohydrate agars were observed for acid color of the indicator after 7 and 28 days of incubation at 28 C.

Decomposition of adenine, hypoxanthine, L-tyrosine, and xanthine. Adenine (0.5 g) was suspended in distilled water (10 ml), autoclaved, mixed thoroughly with 100 ml of sterile nutrient agar (peptone, 5 g; beef extract, 3 g; agar, 15 g; distilled water, 1,000 ml; adjusted to pH 7.0), cooled to 45 C, mixed again, and poured into sterile plates. Care was taken to obtain an even distribution of the adenine throughout the solidified agar. Each culture was streaked once across a plate, incubated at 28 C, and observed at 14 and 21 days for the disappearance of the crystals underneath and around the growth. Except in the use of 0.4 g of xanthine, plates of hypoxanthine, L-tyrosine, and xanthine were prepared, inoculated, and incubated in the same manner.

Decomposition of casein. A 5-g amount of skim milk powder (Difco) suspended in 50 ml of distilled water and 1 g of agar in 50 ml of distilled water were autoclaved separately and cooled to 45 C. The suspensions of milk and agar were then mixed and poured into plates. Each culture was streaked once across a plate and then incubated at 28 C. At 7 and 14 days the cultures were examined for clearing of the casein underneath and around the growth.

A heavy inoculum was used in testing for the decomposition of casein and the four amino acids (above). Some cultures grew well but did not dissolve casein or the crystals of the amino acids except around the larger clumps of inoculum.

Decomposition of urea. A 10-ml amount of a 15% solution of urea sterilized by filtration was added to 75 ml of sterile urease broth (KH_2PO_4 , 10 g;

Na_2HPO_4 , 9.5 g; yeast extract, 1 g; 0.04% solution of phenol red, 20 ml; distilled water, 1,000 ml; adjusted to pH 6.7). The mixture was pipetted aseptically in 2.5-ml amounts into sterile plugged tubes and inoculated with actively growing cultures. An alkaline reaction after 28 days of incubation at 28 C demonstrated the presence of urease.

Determination of diaminopimelic acid and carbohydrates. The presence and form of diaminopimelic acid (DAP) and the presence of carbohydrates in hydrolysates of whole cells were established by the chromatographic methods of Becker et al. (3) and of Lechevalier (15), respectively.

Growth at different temperatures. Slants of yeast dextrose agar (yeast extract, 10 g; glucose, 10 g; agar, 15 g; tap water, 1,000 ml; pH 6.8) were inoculated and quickly heated or cooled to the desired temperature in a water bath. The slants were then placed in a water bath at the same temperature inside a constant-temperature incubator, with water level and temperature of bath being carefully maintained. The cultures were observed for growth after 5 days at 35 C or above and after 3 weeks at 10 C.

Growth in Sabouraud dextrose broth. A tube of Difco Sabouraud dextrose broth (neopeptone, 10 g; glucose, 20 g; distilled water, 1,000 ml; pH 5.7) and a tube of glucose broth were inoculated from a 2- to 4-week-old culture in glucose broth in such a way that the inoculum could not be mistaken later for growth. The tubes were observed for growth after 2 and 4 weeks of incubation at 28 C.

Growth on MacConkey agar. Cultures on slants of MacConkey agar (Difco) were inoculated in the same manner and at the same time as the cultures for the determination of resistance to methyl violet and pyronin B (see below). One control culture served for the three determinations. Growth and color change of the neutral red indicator were noted after 7 and 28 days.

Hydrolysis of esculin. The cultures were inoculated into esculin broth (esculin, 1 g; ferric citrate, 0.5 g; peptone, 10 g; NaCl, 5 g; distilled water, 1,000 ml), incubated at 28 C, and observed for growth and blackening of the medium at 2 and 4 weeks (4). A tube of the same broth without esculin was also inoculated and used as a control.

Hydrolysis of hippurate. The hippurate broth (2) consisted of (grams per liter): tryptone, 10; beef extract, 3; yeast extract, 1; glucose, 1; Na_2HPO_4 , 5; sodium hippurate, 10. After 6 and 8 weeks of incubation, the cultures were tested for benzoic acid by mixing 1 ml of the culture, as free of growth as possible, with 1.5 ml of 50% H_2SO_4 . The appearance of crystals in the acid mixture after 4 h at room temperature indicated hydrolysis of the hippurate.

Hydrolysis of starch. Potato starch (1.5 g) was suspended in cold distilled water (5 to 10 ml) and then added to nutrient agar (100 ml), autoclaved, cooled to 45 C, carefully mixed to suspend the starch throughout the agar, and poured into plates. Duplicate plates were streaked once across with each culture and incubated at 28 C. One plate was flooded with 95% ethanol at 5 days and the other at 10 days. After standing for 15 to 30 min, the unchanged starch

TABLE 1. *History of strains*

Strain no.	Name when received, source, strain name or number
<i>Nocardia autotrophica</i>	
1595 (ATCC 19727)	<i>N. autotrophica</i> (Takamiya and Tubaki) Hirsch; H. G. Schlegel, Univ. Göttingen; P. Hirsch; A. Takamiya
1523	<i>N. brasiliensis</i> ; D. Berd, Center for Disease Control, Atlanta, Ga. (147, parotid gland)
1357	<i>N. caviae</i> (Erikson) Gordon and Mihm; L. K. Georg, Center for Disease Control, Atlanta, Ga. (B 831a, bovine mastitis)
3520	<i>N. coeliaca</i> (Gray and Thornton) Emmart; S. A. Waksman; E. W. Emmart
418	<i>N. coeliaca</i> ; W. C. Haynes, USDA, Peoria, Ill. (NRRL B-1365); W. T. Sokolski; E. W. Emmart
543	<i>N. coeliaca</i> ; E. N. Azarowicz, Univ. of California, Los Angeles; USDA
703	<i>N. coeliaca</i> ; C. W. Emmons, Nat. Inst. of Health, Bethesda, Md. (9964); E. W. Emmart
704	<i>N. coeliaca</i> ; C. W. Emmons (9964A); E. W. Emmart
1337	<i>N. coeliaca</i> ; NCIB (8939); Boots Ltd. (116)
1338	<i>N. coeliaca</i> ; NCIB (9574); R. W. Traxler; U. A. Phillips
1576, 1578	<i>N. coeliaca</i> ; L. K. Georg (W1961, W1963); L. E. Vázquez
1577, 1579	<i>N. coeliaca</i> ; L. K. Georg (W1962, W1995)
1300, 1333-1335	<i>Nocardia</i> sp.; M. P. Lechevalier, Rutgers Univ. (SS 1/1, N11, U4, 7-34; soil)
1309	<i>Nocardia</i> sp.; L. J. Kunz, Massachusetts General Hospital, Boston, Mass. (F 354, clinical specimen)
1446	<i>Nocardia</i> sp.; M. P. Kraus, Univ. of Delaware (RM 66A)
1528	<i>Nocardia</i> sp.; M. P. Lechevalier (G-216, aluminum hydroxide gel)
1597	<i>Nocardia</i> sp.; W. A. Causey, Center for Disease Control, Atlanta, Ga. (N37, spinal fluid)
1598	<i>Nocardia</i> sp.; L. E. Vázquez, Centro Medico de Puerto Rico, San Juan (CM 1512, bronchial washing)
1105	<i>Streptomyces</i> sp.; J. M. Mihm, Rutgers Univ. (eggplant rind)
1124	<i>Streptomyces</i> sp.; L. K. Georg (A588)
1299	<i>Streptomyces</i> sp.; N. F. Conant, Duke Univ. (3300, corneal ulcer)
1320	Unidentified isolation; I. Chapman, City Hospital Center, Elmhurst, N.Y. (8, spinal fluid)
1398	Unidentified isolation; H. Fedukowicz, New York Univ. (TSA)
<i>Nocardia asteroides</i>	
524, 534	<i>N. asteroides</i> (Eppinger) Blanchard; N. F. Conant (2221, lung; 2338, foot sinus)
526-528	<i>N. asteroides</i> ; N. F. Conant (2249, 2250, 2251); B. F. Fetter (130, 142, 8076; abscesses)
530	<i>N. asteroides</i> ; N. F. Conant (2280); J. F. Mohn (brain abscess)
531	<i>N. asteroides</i> ; N. F. Conant (2293); W. B. VandeGrift (pus)
532	<i>N. asteroides</i> ; N. F. Conant (2298); W. E. Bray (hand lesion)
533	<i>N. asteroides</i> ; N. F. Conant (2336); S. B. Salvin (2001)
— ^a	<i>N. asteroides</i> ; J. Rozanis, Western Univ. (10905)
N630	<i>N. blackwellii</i> (Erikson) Waksman and Henrici; NCTC (630); J. McFadyean; J. T. Edwards (hock joint of a foal)
N1935	<i>N. cuniculi</i> (<i>Actinomyces sumatrae</i> Erikson); NCTC (1935); E. P. Snijders (rabbit)
1405	<i>N. pasteuroides</i> ; H. A. Lechevalier; M. Goodfellow (N94); A. Gonzalez-Ochoa (35); Univ. of California, Los Angeles
<i>Nocardia caviae</i> ^b	
402	<i>N. asteroides</i> ; C. W. Emmons (9975)
1233	<i>N. asteroides</i> ; S. G. Bradley, Univ. of Minnesota (92); N. M. McClung; C. W. Emmons (9903)
1259	<i>N. caviae</i> ; F. Mariat (751)
1291	<i>N. caviae</i> ; R. S. Sandu, Univ. of Delhi; F. Mariat (IP 318)

TABLE 1.—Continued

Strain no.	Name when received, source, strain name or number
<i>Nocardia caviae</i> ^b	
1315, 1321	<i>N. caviae</i> ; L. Ajello, Center for Disease Control, Atlanta, Ga. (45-62-66, bronchial washings; 45-323-66, lung of monkey)
1316	<i>N. caviae</i> ; L. N. Mohapatra, All India Inst. of Med. Sci., New Delhi (12, soil)
1342	<i>N. caviae</i> ; I. A. Conti Diaz, Inst. de Higiene, Montevideo (1551, soil)
1355, 1356	<i>N. caviae</i> ; L. Georg (B 812, human lung; 831 b, bovine mastitis)
1365, 1370	<i>N. caviae</i> ; E. L. Biberstein, Univ. of California, Davis (359-3B, sheep lung; dog lung)
1460	<i>N. caviae</i> ; A. Avram, Centrul Dermato-Venerologic, Bucharest (NCl, mycetoma)
1402	<i>N. uniformis</i> Marton and Szabó; H. A. Lechevalier, M. Goodfellow (N3); NCIB (9631)
1203	<i>Nocardia</i> sp.; D. B. Louria, Bellevue Hosp., New York, N. Y.
1237	<i>Nocardia</i> sp.; C. W. Emmons (9988, mycetoma)
1252	<i>Nocardia</i> sp.; R. S. Sandhu (SL 163); P. V. Kurup (soil)
1368	<i>Nocardia</i> sp.; C. McDermont, Dept. of Public Health, Montgomery, Ala. (E-51, sputum)
1522	<i>Nocardia</i> sp.; D. Berd (78, abscess)
1088	<i>Streptomyces albus</i> (Rossi-Doria) Waksman and Henrici; H. D. Tresner, American Cyanamid Co., Pearl River, N. Y. (A0930-H-5187)
1073	<i>Streptomyces</i> sp.; J. B. Routein, Charles Pfizer and Co., Brooklyn, N.Y. (AP 9441, 50393-036)
1351	Unidentified isolation; L. D. Ashburner, Fisheries and Wildlife Dept., Alexandra, Victoria, Australia (rainbow trout)
<i>Nocardia brasiliensis</i>	
1188	<i>N. brasiliensis</i> ; M. A. Gordon, Dept. of Health, Albany, N. Y. (M 4801, lesion on thigh)
1502	<i>N. brasiliensis</i> ; J. H. Seabury, Louisiana State Univ. (Thompson)
1336	<i>Nocardia</i> sp.; M. P. Lechevalier (L 36, soil)
<i>Nocardia madurae</i>	
A25292	<i>N. madurae</i> (Vincent) Blanchard; ATCC (25292A); G. Dementjeva (mycetoma pedis)
1159	<i>Streptomyces</i> sp.; T. H. Stoudt, Merck and Co., Rahway, N.J. (I, "White" group)
<i>Nocardia pelletieri</i>	
1350	<i>N. brasiliensis</i> ; J. H. Seabury (Walker, swelling of right mandible)
1367	<i>N. pelletieri</i> (Laveran) Pinoy; M. Silva-Hutner, College of Physicians and Surgeons, New York, N. Y. (1199.38); M. A. Gordon (47293); N. F. Conant; J. B. Fischer
1352-1354	<i>Streptomyces pelletieri</i> (Laveran) Waksman and Henrici; F. Mariat [IP 729 (red), IP 729 (white variant), IP 375-67; mycetomas]
1441-1445	<i>S. pelletieri</i> ; F. Mariat (IP 372, IP 374, IP 379, IP 381, IP 726)
<i>Nocardia dassonvillei</i>	
1371	<i>N. dassonvillei</i> (Brocq-Rousseau) Liegard and Landrieu; M. P. Lechevalier (Boll 1, cotton boll)
1372	<i>N. dassonvillei</i> ; M. P. Lechevalier (V 116 ET); E. Tejera
1380	<i>N. dassonvillei</i> ; L. Ajello (45-286-68, cow)
3564	<i>Streptomyces</i> sp.; S. A. Waksman (3564); B. S. Walker (ulcerating cancerous lesion)
1524-1526	Unidentified isolations; V. Rangaswamy, Government Tuberculosis Sanatorium, Madras (Madras 1, Madras 2, Madras 3A; pulmonary infections)

^a Strain number not assigned.^b *Nocardia otitidis-caviarum* according to Lessel (Int. J. Syst. Bacteriol. 12: 191-192, 1962).

became white and opaque while a clear zone underneath and around the growth measured the hydrolysis of the starch (13).

Oxidation of glucose. A modification of the Hugh and Leifson (10) test was used to determine whether the cultures utilized glucose oxidatively or fermentatively. The basal medium contained (grams per liter): peptone, 2; NaCl, 5; K_2HPO_4 , 0.3; agar, 3. After the pH of the medium was adjusted to 7.1, 15 ml of a 0.04% solution of bromocresol purple was added. The semisolid agar was tubed (7.5 ml per 16-mm tube), autoclaved, and quickly cooled. Before the agar solidified, 0.5 ml of 15% aqueous solution of glucose, also sterilized by autoclaving, was added to each tube. As soon as the agar solidified, duplicate tubes were inoculated by stabbing from a 2- to 4-week-old culture in glucose broth; one tube was sealed with vaspar (paraffin, 60%; vaseline, 40%). When the vaspar seal occasionally pulled away from the inside of the tube during the first 24 h of incubation at 28 C, the culture was resealed by heating the glass around the seal in a small flame. The cultures were observed at 7 and 28 days. A culture that grew and produced the acid color of the indicator in both tubes was recorded as fermenting glucose; a culture that grew in both tubes but formed the acid color only in the aerobic tube was recorded as oxidizing glucose.

Resistance to bacitracin and penicillin. A tube containing 20 ml of yeast dextrose agar was inoculated with one or two loopfuls of a 14- to 28-day-old culture in glucose broth. The agar was carefully mixed and poured into a sterile plate. After the agar solidified, a 10-unit bacitracin sensitivity test disk (BBL) and a 10-unit penicillin sensitivity disk (Difco) were placed, by using sterile forceps, approximately 3 cm apart on the agar. The plates were incubated at 28 C and observed at 3, 5, and 7 days for a zone of inhibition around the disks. If there was no growth on the plate, duplicate plates were prepared as before; disks were added to one plate, and the second plate, without disks, was incubated as a control.

Resistance to lysozyme. Glycerol broth containing 0.005% lysozyme was prepared by dissolving 0.1 g of lysozyme (Nutritional Biochemicals Corp., Cleveland, Ohio) in 100 ml of distilled water in a volumetric flask and sterilizing the solution by filtration. A 5-ml amount of the lysozyme solution was mixed with 95 ml of sterile glycerol broth (peptone, 5 g; beef extract, 3 g; glycerol, 70 ml; distilled water, 1,000 ml; pH 7.0) and dispensed aseptically in 2.5-ml amounts in sterile plugged tubes. A small loopful of a 14- to 30-day-old culture in glucose or glycerol broth was inoculated into a tube of lysozyme broth and another loopful was inoculated into a tube of glycerol broth so that later the inoculum could not be mistaken for growth. After 14 and 28 days of incubation at 28 C, the cultures were observed for growth.

Resistance to methyl violet and pyronin B. Stock aqueous solutions of methyl violet and pyronin B (Matheson, Coleman, and Bell, Norwood, Ohio) were prepared by dissolving enough of the dye to result in a 1% solution of total dye content. Slants of blood agar base (Difco) containing 0.01% of the dye and 70 ml of glycerol per liter were inoculated with a small loop (2.5 mm in outside diameter) from a 14- to 30-day-old

culture in glucose broth. Slants of the same agar without the dye were also inoculated as controls. The cultures were examined for growth after 7 and 28 days at 28 C.

Survival at 50 C. The cultures were inoculated on slants of yeast dextrose agar. The slants were heated to 50 C in a water bath and then transferred to a water bath at the same temperature inside a constant-temperature incubator. After 8 h the cultures were quickly cooled, incubated at 28 C for 3 weeks, and then examined for growth.

Utilization of benzoic, citric, and other organic acids. Modifications of Koser's (14) citrate agar were prepared by adding 2 g (or equivalent) of either sodium benzoate, sodium citrate, sodium lactate, calcium malate, mucic acid, sodium oxalate, or sodium succinate to: NaCl, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $(NH_4)_2HPO_4$, 1.0 g; KH_2PO_4 , 0.5 g; agar, 15 g; distilled water, 1,000 ml; 0.04% solution of phenol red, 20 ml. The pH value of the malate agar was adjusted to 7.2 with 1 N NaOH; the pH value of the other agars was adjusted to 6.8 before autoclaving. Slants of the seven agars were inoculated and incubated at 28 C. Utilization of the organic acids was established by the alkaline color of the phenol red after 28 days.

RESULTS

Nocardia coeliaca. After 18 h of incubation at 28 C, cultures of Gray and Thornton's strain of *M. coeliacum* (ATCC 17041 and Skerman 132) grown on yeast dextrose agar were composed of gram-positive, pleomorphic rods, coccoid forms, and short filaments. At 5 days, less than 1% of the cells grown on glycerol agar were acid fast. Colonies on tap-water agar were dense and lobate, and nearly all had smooth margins. Filamentous colonies and aerial hyphae were not formed. When observed under a microscope the duplicate strains, therefore, resembled typical strains of *M. rhodochrous* (6).

Hydrolysates of whole cells of ATCC strain 17041 contained *meso*-DAP, arabinose, galactose, and mannose. Glucose, madurose (16), and ribose were not present. This strain conformed, therefore, to the strains of *M. rhodochrous* we have examined chromatographically. All of these (34 strains) had *meso*-DAP, and 15 of the 34 (the other 19 strains were not tested for carbohydrates) contained arabinose, galactose, and mannose. Madurose was not observed, and the presence of glucose and ribose was variable (Table 2).

Except for a weak action on tyrosine by Skerman's strain 132, physiological reactions of the duplicate strains (Table 3) were identical and in good agreement with the reactions of typical strains of *M. rhodochrous*. Positive

TABLE 2. Carbohydrates in whole-cell hydrolysates of *M. rhodochrous*^a

Strain no.	Arabinose	Galactose	Glucose	Mannose	Madurose	Ribose
329	+	+	tr	+	—	—
333	+	+	tr	+	—	—
338	+	+	+	+	—	tr
343	+	+	—	+	—	—
625	+	+	—	+	—	—
1339	+	+	+	+	—	tr
1340	+	+	+	+	—	—
3407	+	+	—	+	—	tr
3408	+	+	+	+	—	tr
NCTC 576	+	+	+	+	—	tr
ATCC 19140	+	+	—	+	—	—
Faur 861	+	+	—	+	—	tr
Feeley SPU 2301	+	+	+	+	—	tr
Little 308	+	+	—	+	—	—
Seibert P4600	+	+	+	+	—	tr

^a +, Carbohydrate present; —, not present; tr, trace amount only.

characteristics (85 to 100% of 97 strains positive) were: hydrolysis of starch; growth at 35, 28, and 10 C; acid formation from glucose, mannitol, mannose, sorbitol, and trehalose; utilization of citrate, lactate, malate, and succinate; and oxidation of glucose. Negative properties were: decomposition of casein, hypoxanthine, and xanthine; growth at 52 C; acid formation from arabinose, dulcitol, erythritol, lactose, melibiose, α -methyl-D-glucoside, raffinose, and rhamnose; utilization of mucate and oxalate; resistance to methyl violet, pyronin, bacitracin, and penicillin; color change of MacConkey agar; hydrolysis of hippurate; and deamination of phenylalanine. The duplicate strains formed acid from inositol and, in this respect, were like 21% of the 97 strains of *M. rhodochrous* described by Gordon (6).

Nocardia autotrophica. Although the nocardin strain and some of the other strains like it in our collection (listed under *N. autotrophica* in Table 1) had been cultivated in vitro for 25 years, the yellowish or brownish growth of the cultures on yeast dextrose agar and other media was still thickly coated with whitish aerial hyphae. Cultures on agar slants often exhibited, at the bottom of the slant, a characteristic patch of brown wrinkled growth that was more sparsely covered with aerial hyphae.

After 5 days on glycerol agar, some cultures consisted of long, branching filaments that remained intact during the preparation and staining of the smear. In other cultures,

however, the branching filaments were of varying length and some had fragmented into rods and coccoid forms. Cultures of the 28 strains of *N. autotrophica* (Table 1) were not acid fast. Cultures grown on yeast dextrose agar for 2 days were gram positive. The 28 strains produced densely or loosely filamentous colonies that were soon covered with an abundance of aerial hyphae. The aerial hyphae of most strains were long, straight, and branching, whereas those of other strains were wavy or curled in loose spirals. The aerial hyphae of 37% of the cultures formed chains of cylindrical spores (conidia).

Hydrolysates of whole cells of the 28 strains contained *meso*-DAP, galactose and equal or greater amounts of arabinose, and mannose. Glucose was usually present; ribose was present in 50% of the cultures; and madurose was absent.

A pattern of physiological reactions for distinguishing the 28 strains of *N. autotrophica* is presented in Tables 4 and 5. In Table 4 the physiological properties observed of four replicates of Emmart's nocardin strain (strains 418, 703, 704, and 3520) are compared with the reactions of the type strain of *N. autotrophica* (IMRU 1595, also ATCC 19727) and with those of a typical or representative strain of the 28 strains of *N. autotrophica* in our collection. The four replicates varied among themselves in four of their properties (decomposition of tyrosine, survival at 50 C for 8 h, reduction of nitrate to nitrite, and acid production from α -methyl-D-glucoside), and the type strain of *N. autotrophica* differed from the replicates of the nocardin strain in three (decomposition of xanthine, acid production from inositol, and growth in Sabouraud dextrose broth). With the exception of decomposition of tyrosine, these seven reactions were variable for the 28 strains of *N. autotrophica*.

Positive physiological properties for distinguishing the 28 strains of *N. autotrophica* (85 to 100% of strains positive) from the six previously described (7) *Nocardia* species represented in our collection were: decomposition of adenine, hypoxanthine, tyrosine, and urea; utilization of citrate, lactate, malate, and succinate; hydrolysis of starch and esculin; and acid formation from adonitol, erythritol, galactose, glucose, maltose, mannitol, mannose, sorbitol, and xylose (Table 5). Negative properties (0 to 14% of strains positive) in this pattern of reactions were: decomposition of casein; growth at 45 C; utilization of mucate and oxalate; resistance to bacitracin, lysozyme, and penicillin; and formation of acid from dulcitol, lactose, melibiose, raffinose, and rhamnose.

TABLE 3. *Some physiological characteristics of M. rhodochrous*

Property	<i>M. rhodochrous</i>		Type strain of <i>N. coeliaca</i>	
	97 strains (% positive)	Typical strain	ATCC 17041	Skerman 132
Decomposition of				
Casein	0	—	—	—
Hypoxanthine	2	—	—	—
Tyrosine	74	+ or —	—	±
Xanthine	0	—	—	—
Hydrolysis of starch	97	+	+	+
Growth at				
52 C	3	—	—	—
45 C	46	+ or —	—	—
40 C	70	+ or —	—	—
35 C	91	+	+	+
28 C	100	+	+	+
10 C	100	+	+	+
Acid from				
1 (+) Arabinose	8	—	—	—
Dulcitol	0	—	—	—
i-Erythritol	7	—	—	—
Glucose	99	+	+	+
i-Inositol	21	+ or —	+	+
d (+) Lactose	0	—	—	—
d (—) Mannitol	99	+	+	+
d (+) Mannose	99	+	+	+
d (+) Melibiose	0	—	—	—
α-Methyl-D-glucoside	0	—	—	—
d (+) Raffinose	0	—	—	—
1 (+) Rhamnose	9	—	—	—
d-Sorbitol	100	+	+	+
d (+) Trehalose	97	+	+	+
Utilization of				
Benzoate	70	+ or —	—	—
Citrate	90	+	+	+
Lactate	100	+	+	+
Malate	99	+	+	+
Mucate	0	—	—	—
Oxalate	0	—	—	—
Succinate	100	+	+	+
Resistance to				
Methyl violet	0	—	—	—
Pyronin	0	—	—	—
Bacitracin	0 ^a	—	—	—
Penicillin	6	—	—	—
Color change of MacConkey agar	0	—	—	—
Hydrolysis of hippurate	9	—	—	—
Deamination of phenylalanine	12	—	—	—
Oxidation of glucose	99	+	+	+

^a Fifty-two strains examined.

Although not all the physiological tests were applied to our strains of *M. rhodochrous* (Table 3), decomposition of hypoxanthine (92% of the strains of *N. autotrophica* positive) and production of acid from erythritol (100% positive), in addition to the morphological features, separ-

ated the strains of *N. autotrophica* from strains of the rhodochrous complex. To a lesser extent, decomposition of xanthine (79% of the strains positive) and acid formation from arabinose (82% positive) were helpful in dividing the two taxa.

DISCUSSION

ATCC 17041, one of Gray and Thornton's (8) original isolates of *M. coeliacum*, now known as *Nocardia coeliaca*, has been designated herein as the type strain of *N. coeliaca*.

Because the nocardin-producing strain of Emmart (5) and others like it form filamentous colonies and an abundance of aerial hyphae, Gray and Thornton, and Jensen (11, 12) would not have assigned them to the genus *Mycobacterium*. The morphological separation of the

TABLE 4. Some properties of the nocardin-producing strain and of strains of *N. autotrophica*

Property	Nocardin strain				<i>N. autotrophica</i>	
	418	703	704	3520	Type strain	Typical strain
Acid fastness	—	—	—	—	—	—
Decomposition of						
Adenine	+	+	+	+	+	+
Casein	—	—	—	—	—	—
Hypoxanthine	+	+	+	+	+	+
Tyrosine	+	—	+	+	+	+
Urea	+	+	+	+	+	+
Xanthine	+	+	+	+	—	+ or —
Growth at						
45 C	—	—	—	—	—	—
10 C	+	+	+	+	+	+ or —
Survival at 50 C for 8 h	—	—	—	+	—	+ or —
Nitrate from nitrite	+	+	—	+	+	+ or —
Acid from						
Adonitol	+	+	+	+	+	+
1 (+) Arabinose	+	+	+	+	+	+ or —
Dulcitol	—	—	—	—	—	—
i-Erythritol	+	+	+	+	+	+
d (+) Galactose	+	+	+	+	+	+
Glucose	+	+	+	+	+	+
i-Inositol	—	—	—	—	+	+ or —
d (+) Lactose	—	—	—	—	—	—
d (+) Maltose	+	+	+	+	+	+
d (—) Mannitol	+	+	+	+	+	+
d (+) Mannose	+	+	+	+	+	+
d (+) Melibiose	—	—	—	—	—	—
α -Methyl-D-glucoside	+	—	+	+	—	+ or —
d (+) Raffinose	—	—	—	—	—	—
1 (+) Rhamnose	—	—	—	—	—	—
d-Sorbitol	+	+	+	+	+	+
d (+) Xylose	+	+	+	+	+	+
Utilization of						
Benzoate	—	—	—	—	—	—
Citrate	+	+	+	+	+	+
Lactate	+	+	+	+	+	+
Malate	+	+	+	+	+	+
Mucate	—	—	—	—	—	—
Oxalate	—	—	—	—	—	—
Succinate	+	+	+	+	+	+
Resistance to						
Bacitracin	—	—	—	—	—	—
Lysozyme	—	—	—	—	—	—
Penicillin	—	—	—	—	—	—
Hydrolysis of						
Esculin	+	+	+	+	+	+
Hippurate	—	—	—	—	—	+ or —
Starch	+	+	+	+	+	+
Growth in Sabouraud dextrose broth	+	+	+	+	—	+ or —

TABLE 5. *Some properties of seven species of Nocardia*

Property	Positive strains (%)						
	<i>N. aster- oides</i> (137) ^a	<i>N. caviae</i> (37)	<i>N. brasil- iensis</i> (65)	<i>N. auto- trophica</i> (28)	<i>N. madurae</i> (46)	<i>N. pelle- tieri</i> (25)	<i>N. dasson- villei</i> (33)
Acid fastness	60	62	82	0	0	0	0
Decomposition of							
Adenine	0	3	3	92	0	0	100
Casein	0	0	98	0	98	100	97
Hypoxanthine	4	100	95	92	98	76	100
Tyrosine	1	5	100	89	93	100	100
Urea	96	100	100	86	0	0	36
Xanthine	0	100	0	79	0	0	100
Growth at							
45 C	41	38	2	0	35	68	0
10 C	16	24	38	82	0	0	0
Survival at 50 C for 8 h	96	97	2	64	100	100	97
Nitrite from nitrate	91	97	91	54	98	100	91
Utilization of							
Benzoate	0	0	0	36	7	0	0
Citrate	34	38	100	96	83	0	97
Lactate	46 ^b	62	100	100	98	24	100
Malate	96	100	100	100	7	52	100
Mucate		5		0		0	0
Oxalate		0	0 ^c	4	0	0	0
Succinate	95	100	100	100	83	0	100
Resistance to							
Bacitracin				0			
Lysozyme	100	100	100	0	9	0	0
Penicillin				0			
Hydrolysis of							
Esculin	100 ^c	100	100 ^c	89	98	0	9
Hippurate				18	4	0	100
Starch	69	51	60	100	100	0	100
Acid from							
Adonitol	1	3	0	92	91	0	0
1 (+) Arabinose	0	14	0	82	100	0	58
Dulcitol	0 ^b	0	0 ^c	0	0	0	0
i-Erythritol	5	0	0	100	0	0	0
d (+) Galactose	18	8	95	96	87	0	58
Glucose	99	100	98	100	100	100	100
i-Inositol	3	100	100	32	61	0	0
d (+) Lactose	0	0	0	0	55	0	0
d (+) Maltose	0	8	3	85	55	0	100
d (-) Mannitol	1	86	95	100	100	0	100
d (+) Mannose	16	35	69	100	93	0	94
d (+) Melibiose	0 ^b	0	0 ^c	0	0	0	9
α-Methyl-D-glucoside	0	0	0	43	0	0	24
d (+) Raffinose	0	0	0	0	0	0	0
1 (+) Rhamnose	32	3	3	14	100	0	55
d-Sorbitol	0	3	0	96	0	0	0
d (+) Xylose	0	5	2	100	100	0	70
Growth in Sabouraud dextrose broth	94 ^b	97	96 ^c	75	15	0	3

^a Numbers in parentheses indicate the number of strains examined.^b Ninety strains examined.^c Fifty strains examined.

nocardin strains from the type strain of *N. coeliaca* is supported by physiological criteria and, according to the examination of a limited number of strains, by their lipids. Lechevalier, Horan, and Lechevalier (17) reported that eight strains of the rhodochrous complex, to which the type strain of *N. coeliaca* belongs, contained nocardomycolic acids, whereas four nocardin strains (1105, 1124, 1320, 1337) had no nocardomycolic or mycolic acids. We are convinced, therefore, that the nocardin strains are mistakenly labeled *N. coeliaca*.

Among the strains in our collection like Emmart's nocardin-producing strain, the type strain of *N. autotrophica* is the only one providing a name for the species we believe to be distinct. The widespread distribution of the nocardin strains under the name *N. coeliaca* is unfortunate, but a complete elimination of the mislabeling should be pursued no matter how discouraging.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant AI-06276 from the National Institute of Allergy and Infectious Diseases. We are grateful for this support and for the assistance of the investigators who sent us their strains.

REPRINT REQUESTS

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