Enrichment, Isolation, and Emended Description of *Pedomicrobium ferrugineum* Aristovskaya and *Pedomicrobium manganicum* Aristovskaya

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In 1961 Aristovskaya described the new hyphal budding bacteria *Pedomicrobium ferrugineum* and *Pedomicrobium manganicum*. Only a limited amount of information concerning these bacteria was obtained from enrichment cultures, and these cultures no longer exist. In an effort to revive investigations on pedomicrobia, six new iron-depositing strains were isolated from podzolic soils. One of these isolates, strain S-1290 (=ATCC 33119 =DSM 1540), was investigated thoroughly and is cited in the Approved Lists of Bacterial Names as the type strain of *P. ferrugineum*. Four other isolates (strains P-1196, Q-1197, R-1198, and T-1130) showed sufficient similarity to strain S-1290 to be placed in the same species. The taxonomic position of the sixth isolate, strain F-1225, is not yet certain. One additional isolate, strain E-1129 (=ATCC 33121 =DSM 1545), which deposits manganese, was isolated from a quartzite rock pool. This isolate closely resembles the original description of *P. manganicum* and is cited in the Approved Lists as the type strain of this species. I present an emended description of the genus *Pedomicrobium* and compare the generic properties of *Pedomicrobium* with those of other hyphal budding bacteria. To encourage further work on pedomicrobia, I also describe enrichment and isolation procedures for these bacteria. Cultures of all of the new strains are available from the American Type Culture Collection and from the Deutsche Sammlung von Mikroorganismen.

In 1961, Aristovskaya (1) described budding bacteria with threadlike outgrowths (hyphae); these organisms were found predominantly in podzolic soils in the Karelian Isthmus, USSR. Enrichment cultures on agar media containing "organomineral complexes of fulvic acids" served as bases for descriptions of these new bacteria, which were named *Pedomicrobium ferrugineum* and *Pedomicrobium manganicum* because they were able to accumulate oxidized iron and manganese compounds, respectively, on their cell surfaces.

Subsequently (2), a third species "*Pedomicrobium podsolicum*" was described (names in quotation marks are not on the Approved Lists of Bacterial Names [35]; hence, they are without standing in nomenclature). This organism was characterized by the ability to deposit both iron and manganese oxides. After further investigations, Aristovskaya and Zavarzin (5) reported the oxidation of iron by *Pedomicrobium* cultures when they were grown on media containing "organoiron compounds of humic acids."

Khak-mun (18, 19) found pedomicrobia morphologically similar to *P. manganicum* in manganese-rich, peaty, brown forest soils, as well as in manganese crusts on shale and limonite in

Sakhalin, USSR. He described oval cell types ("*Pedomicrobium manganicum* subsp. *sachalinicum*") and other cells which were spherical; both types accumulated oxidized manganese compounds.

The aquatic environment is also a habitat of *Pedomicrobium*-like organisms. Hirsch (13) isolated several iron-depositing pedomicrobia from seawater, and Hirsch and Rheinheimer (16) used seawater samples containing 2.5% salt for enrichment cultures of other *Pedomicrobium* strains. Kutuzova (20) found pedomicrobia in the sediment of a freshwater lake. Tyler and co-workers isolated *Pedomicrobium*-like bacteria, described as *Hyphomicrobium* strains, from hydroelectric pipelines in Tasmania, Australia (6, 7, 40-43). These organisms caused thick manganese deposits in various freshwater pipelines in England, Norway, Australia, and New Zealand (39).

In all of these reports on pedomicrobia, taxonomically useful data were sparse. To date, pedomicrobia can be identified only by the reproductive process (bud formation), by the number of hyphae formed, and by the ability to cause heavy metal depositions. It seems quite likely that, using these characteristics, workers have brought together bacteria which are different in

other respects and have classified them together in this genus, which at present is not well defined.

Since the original enrichment cultures have been lost (Aristovskaya, personal communication), Gebers and Hirsch attempted to isolate new *Pedomicrobium* strains which were as similar to the original cultures as possible (see below; R. Gebers and P. Hirsch, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, N34, p. 190). Two of the three original species, *P. ferrugineum* (including five strains) and *P. manganicum* (represented so far by one strain), were included in the recently published Approved Lists of Bacterial Names (35); as cited in the Approved Lists, strains S-1290 (=ATCC 33199 =DSM 1540) and E-1129 (=ATCC 33121 =DSM 1545) are the respective type strains of these two species. The third species, "*P. podsolicum*," was not included in the Approved Lists because no strain sufficiently fitting its original description has been discovered.

One purpose of this paper is to present a simple, reproducible method for the enrichment and isolation of pedomicrobia from soil. Also included is an emended description of the genus *Pedomicrobium* based on pure-culture investigations with the new isolates.

MATERIALS AND METHODS

Media. For enrichment and isolation, I used humic gel agar, which was modified from the medium of Aristovskaya (1). This medium contained 5 g (wet weight) of humic gel (fulvic acid iron sesquioxide complexes) per liter and 18 g of agar (Difco Laboratories, Detroit, Mich.) per liter, and its pH was 5.7. Humic extracts were prepared by the method of Ponomareva (31) from mixed samples of A_n, E, B_n, and B_{tc} horizons of podzolic soils (11). Peptone actidion agar, which was used in enrichment and isolation procedures, contained (per liter) 1 g of D-ribose, 0.5 g of peptone (Difco), and 20 ml of Hutner mineral salts solution (8). A sterile, filtered vitamin solution (37) (final concentration, 1%, vol/vol) and sterile actidion (final concentration, 0.015%, wt/vol; Roth, Darmstadt, Germany) were added after autoclaving; the pH of this medium was 5.7. *Pedomicrobium* standard medium (PSM), which supported the growth of all isolates, contained (per liter) 10 mM sodium acetate, 0.5 g of yeast extract (Difco), 1 ml of "metals 44" (8), and 10 ml of a vitamin mixture (37). The pH of this medium was adjusted to 9.0. PSM was sterilized by autoclaving (pH 7.0) or filtering (pH 8.5 to 9.0). If desired, 18 g of agar (Difco) was added before autoclaving.

Growth conditions. Enrichment cultures were incubated at either 20 or 30°C; pure cultures were always incubated at 30°C. Cultures on solid media were placed in plastic bags to prevent desiccation during long incubation periods. Liquid cultures were aerated by magnetic stirring or shaking; in addition, volumes

of more than 300 ml were gassed with forced sterile air. Cultures were usually incubated in the dark.

Enrichment and isolation. Samples (5 g, wet weight) of podzols from northern Germany were suspended in 500 ml of sterile 0.85% (wt/vol) NaCl and then streaked onto solid media (humic gel agar and peptone actidione agar). After prolonged incubation (3 to 9 months), one to three colonies containing pedomicrobia were suspended carefully in 0.5 ml of a sterile saline solution with a Potter-Elvehjem glass homogenizer and then streaked onto humic gel agar and peptone actidione agar. These and subsequent isolation plates were incubated for 20 to 60 days until visible colonies developed.

An enrichment culture obtained from a water sample from a quartzite rock pool (Opferkessel) in the Park of Fontainebleau, France, was kindly provided by F. E. W. Eckhardt, Kiel, Germany. When this culture was plated onto mineral salts medium containing 0.1% peptone, tiny *Pedomicrobium* colonies developed, which could be isolated by the technique described above.

Preservation of pure cultures. Pedomicrobia grown on autoclaved PSM agar slants in screw-capped tubes survived at incubation temperatures of 30°C for more than 2 years. For longer periods of preservation, 1 part of a liquid culture was mixed with 1 part of sterilized milk (fat content, 0.3%, dry weight) and freeze-dried at -55°C.

Microscopy. The methods used for microscopic observations of these organisms have been described previously (11). To prepare thin sections, the following methods (12a, 17) were modified. Cells from the logarithmic stage of growth were prefixed by adding glutaraldehyde (final concentration, 0.5%, vol/vol) and 0.1 M CaCl₂ (final concentration, 0.01 M) to the culture. After 30 min, the cell suspensions were centrifuged at 16,000 × g for 20 min and washed twice with 10-fold-diluted Veronal acetate buffer (17) containing 0.01 M CaCl₂. The resulting pellets were each divided into two fractions and then suspended in 3 ml of diluted buffer to which 0.1% (wt/vol) OsO₄ had been added. For one of the two fractions, the buffer contained 0.05% (wt/vol) ruthenium red. After 3 h of fixation, the cells were centrifuged and embedded in 2% water agar. Agar blocks were postfixed for 1 h with 0.1% OsO₄ and then treated for 2 h with 0.5% uranyl acetate, dehydrated in ethanol, and embedded in Spurr epoxy resin (36). Polymerization was carried out at 70°C for 8 h. Thin sections were cut with an LKB ultramicrotome III equipped with a diamond knife. Final contrast of the sections was achieved by a 3-min treatment with 1.5% uranyl acetate in 70% methanol, followed by a 3-min treatment with lead citrate (32). Transmission electron microscopy was performed with a Philips EM-300 electron microscope; pictures were taken on Kodak Electron Microscope Film 4489.

Cellular structures. Gram staining of heat-fixed cells and catalase detection were performed as described by Skerman (34). Fe(III) was detected by the Prussian blue reaction. Mn(III, IV) was identified with the benzidine reagent (10), and 5 min of staining with Loeffler methylene blue (34) was used to detect polyphosphate granules. Poly-β-hydroxybutyric acid was

extracted with CHCl_3 at 40°C and was identified by absorption changes at 235 nm after 15 min of boiling in concentrated H_2SO_4 (21). To extract deoxyribonucleic acid (DNA), 0.5 to 1.0 g (wet weight) of cells was suspended in 10 ml of 0.05 M tris(hydroxymethyl)aminomethane (pH 8) containing 0.01 M $\text{MgCl}_2 \cdot \text{H}_2\text{O}$ and 0.01 M KCl; after 2 h of treatment with 100 μg of lysozyme per ml at 37°C , self-digested pronase E (100 $\mu\text{g}/\text{ml}$) and 0.2% (wt/vol) sodium dodecyl sulfate were added, and the preparation was incubated for another 3 h at 37°C . Then the concentration of sodium dodecyl sulfate was increased to 2% simultaneously with an increase in the temperature to 60°C . After 4 h, the DNA was prepared from the lysate by the method of Marmur (26), and the base ratio was determined by thermal denaturation (T_m) values by the method of Mandel et al. (25).

Physiology. Data on the physiological properties of pedomicrobia were obtained by serial tests in liquid media. At least three parallel cultures were checked for each of the growth parameters by determining the increase in cellular protein by the method of Lowry et al. (23). When possible, all media were sterilized by filtration; paraffin was autoclaved before it was added to the media. The effects of antibiotics were studied by the plate diffusion method (28); autoclaved PSM agar plates were inoculated homogeneously, and for each test a central hole was filled with 0.4 ml of a solution containing 100 μg of antibiotic per ml. All antibiotics were used at their optimum pH values; the pH of the agar medium was 6.6. After 6 and 11 days of incubation, the growth inhibition zone was measured.

RESULTS

The origins of the samples used and some of the details of the enrichment procedure have been described previously (11). Both of the agar media used for enrichment cultures, humic gel agar and peptone actidione agar, were suitably selective for pedomicrobia. During the first few weeks of incubation, various other bacteria and fungi (and sometimes algae and amoebae) developed rapidly. After the growth of most of these other organisms declined, pedomicrobia developed. On humic gel agar only a few contaminating microorganisms grew besides the pedomicrobia. Peptone actidione agar allowed faster growth of pedomicrobia and prevented growth of many eucaryotes. After four to eight subcultures on humic gel agar or peptone actidione agar, pure colonies of pedomicrobia were obtained (Table 1). Six *Pedomicrobium* strains were isolated from the podzol samples; all of these accumulated iron oxides but did not accumulate manganese oxides. The one enrichment culture from the quartzite rock pool resulted in the isolation of a manganese oxide-depositing strain.

One representative of the iron-depositing isolates, strain S-1290, was investigated with respect to 138 properties. Subsequently, the re-

TABLE 1. *Origins and strain numbers of the Pedomicrobium isolates used in this study*

Origin	Strain no.			
	(11)	IFAM ^a	DSM ^b	ATCC ^c
Northern Germany podzol	P-111	P-1196	1541	33116
	Q-112	Q-1197	1542	33117
	R-121	R-1198	1543	33118
	S-122	S-1290	1540	33119
	T-123	T-1130	1544	33120
	F-311	F-1225	1546	33122
Quartzite rock pool (France)	E-211	E-1129	1545	33121

^a IFAM, Institut für Allgemeine Mikrobiologie, Universität Kiel, Kiel, Germany.

^b DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Germany.

^c ATCC, American Type Culture Collection, Rockville, Md.

maining iron-depositing strains (P-1196, Q-1197, R-1198, and T-1130), as well as the manganese-depositing strain (E-1129), were checked for the presence of the main properties studied in strain S-1290. Strain F-1225 was investigated only for morphological features.

Properties of *Pedomicrobium* S-1290. (i) Morphology. The cells of strain S-1290 were ovals short rods, or bean shaped (Fig. 1), or sometimes tetrahedral (not shown here); they measured up to 1.2 by 1.8 μm (Fig. 1). Up to three filamentous cellular outgrowths (hyphae) 0.2 μm in diameter were observed per cell; these varied in length with cultural conditions. At least one hypha grew out laterally; the others originated polarly or subpolarly. True branching of the hyphae occurred. Multiplication was by budding at the hyphal tips. Mature buds either separated from their mother hyphae as subpolarly flagellated swimmers (Fig. 2) or remained attached. Intercalary buds were also observed (Fig. 3); pili were lacking.

(ii) Morphology of the colonies. Two types of colonies developed on solid media (Fig. 4). Both types were round with even or radially frayed edges and yellowish red to dark brown in color. Type 1 colonies were large (i.e., 4 mm in diameter) after 10 days on autoclaved PSM, convex, and soft; concentric rings were observed. Type 2 colonies were small (i.e., 1.5 mm in diameter) after 10 days on autoclaved PSM; their surfaces were flat and even or crateriform (bent downward into the agar). Cells in the centers of the colonies were often lysed, giving the colonies a granular appearance. Type 2 colonies had a cartilaginous consistency and could be removed from the agar intact. Pure cultures of the two colony types could not be obtained by streaking onto solid media; when this was done, both large and small colonies produced mixtures of large and small types. Continuous

FIG. 1. *P. ferrugineum* S-1290. This edge of a colony shows aggregated mother cells and the hyphal network. Some hyphal tips exhibit bud formation at various stages (arrows). Phase-contrast micrograph of living cells. Bar = 10 μ m.

FIG. 2. *P. ferrugineum* S-1290 daughter cells at different developmental stages. Cell A, Swarmer cell with polarly inserted flagellum; shows site (nose) of separation from mother hyphae (arrow); cell B, young cell, after losing its flagellum, starts growing a new hypha at the former separation nose (arrow). The dark spots within each of these cells indicate accumulations of storage material. Transmission electron microscope micrograph (H. Völker), Pt/C shadowed. Bar = 1 μ m.

FIG. 3. *P. ferrugineum* S-1290. Diverging from the usual generation cycle, immature buds sometimes remain attached to their mother hyphae. These buds have reached approximately one-half the size of a mature daughter cell. Nevertheless, outgrowth of new hyphae continues. An intercalary hyphal swelling (arrow) indicates branching of the hypha or formation of an intercalary bud. Transmission electron microscope micrograph, negatively stained. Bar = 0.5 µm.

FIG. 4. Two types of *P. ferrugineum* S-1290 colony grown on PSM agar: type 1 (large, convex, smooth) and type 2 (small, flat, cartilaginous). Bar = 0.5 µm.

FIG. 5. Thin section of a *P. ferrugineum* S-1290 cell with a lateral hypha. Note the lack of a septum within the hypha. Modified Kellenberger fixation, transmission electron microscope micrograph (W. C. Ghiorse). Bar = 0.5 μ m.

subculturing of this isolate in PSM led to a step-by-step enrichment of the large (type 1) colonies.

(iii) Cellular structure and composition.

Generally, the cells were gram negative (Fig. 5), although older cells were often gram variable. Extracellular polymers stained with ruthenium red. In the presence of fulvic acid iron complexes, elemental iron powder, FeS, or iron paper clips, oxidized iron compounds were deposited on the surfaces of mother cells and hyphae but not on young buds (11). Each cell contained up to three poly- β -hydroxybutyric acid storage granules; small polyphosphate granules also occurred. Liquid cultures grown in the dark were yellowish white; those grown in artificial light (220 ft-c [2,200 lx]) were reddish orange due to pigments which so far have not been identified. These pigments could be extracted with petrol benzene, methanol, or chloroform. The main absorption maxima of all extracts were observed within the ultraviolet range of 213 to 314 nm; small peaks of the petrol benzene and methanol extracts were found at 462 and 474 nm, respectively. Extracts from cells grown in the light revealed higher absorption values at all wavelengths except for the 474-nm peak, which appeared only in extracts from dark-grown cells. The addition of concentrated H₂SO₄ or 2 N NaOH did not cause any change in the color of the extracts.

TABLE 2. DNA base compositions of *Pedomicrobium* strains

Strain	G + C content (mol%) ^a
S-1290	65.3 $\pm$ 0.4 ^b
P-1196	66.0 $\pm$ 0.3
Q-1197	65.2 $\pm$ 0.4
R-1198	64.5 $\pm$ 0.2
T-1130	65.6 $\pm$ 0.2
E-1129	64.7 $\pm$ 0.2
F-1225	64.9 $\pm$ 0.3

^a The guanine-plus-cytosine (G + C) content was calculated by using the following formula: $G + C = [(T_m \text{ in } 0.1 \times \text{SSC})/50.2] - 0.990$, where $0.1 \times \text{SSC}$ was 0.015 M NaCl plus 0.0015 M sodium citrate (pH 7.0).

^b Mean $\pm$ standard deviation of at least six T_m determinations.

Table 2 shows the DNA base composition of strain S-1290.

(iv) Physiological properties. This organism was microaerophilic to aerobic (growth occurred with 1 to 21% pO₂) and catalase positive. Growth was observed in aerobic atmospheres containing 0.03 or 5% pCO₂. The temperature range for growth was 10 to 40°C; optimum growth occurred at 30°C. The pH range for growth was 3.5 to 10, with optimum growth occurring at pH 9.0.

This organism was heterotrophic and showed

TABLE 3. *Growth of Pedomicrobium S-1290 with various carbon sources*^a

Carbon source	Growth ^b
Acetate	+
Caproate	+
Succinate	+
Fumarate	+
Malate	+
Gluconate	+
Pyruvate	+
Propanol	+
Phenol	+
Glutamate	+
Oxalate	±
Lactate	±
Tartrate	±
Citrate	±
Methanol	±
Ethanol	±
Glycerol	±
Mannitol	±
Cholesterol	±
Paraffin	±
Aspartate	±
Tryptophane	±
Alanine	±
Ribose	—
Glucose	—
Galactose	—
Fructose	—
Maltose	—
Lactose	—
Sucrose	—
Formate	—
Phenylalanine	—
Serine	—
Lysine	—

^a Growth after 9 days of incubation was calculated from the protein yield per milliliter of culture; protein yields were determined by the method of Lowry et al. (23). All carbon sources were tested at a concentration of 10 mM, except paraffin, which was tested at a concentration of 0.3% (wt/vol). Amino acids were used simultaneously as nitrogen sources.

^b +, Good growth (protein yield of >10 µg/ml); ±, poor growth (protein yield of 0.2 to 10 µg/ml); —, no growth.

slow, poor growth with 0.1 to 1% fulvic acid iron sesquioxide complexes as sole carbon and nitrogen sources. Tables 3 and 4 show the utilization of other carbon and nitrogen sources.

Vitamin mixtures (37) stimulated growth, but no single vitamin (e.g., biotin, folic acid, thiamin, calcium pantothenate, cobalamin, riboflavin, nicotinic acid, *p*-aminobenzoic acid, or pyridoxin) was essential. The absence of vitamins led to cell pleomorphism (Fig. 6); subsequent addition of the vitamin mixture reestablished normal cell shape.

Mineral salts in low concentrations, such as metals 44 (8), stimulated growth, but the pres-

ence of these salts was not required for growth. Nitrilotriacetic acid, a chelating ingredient of Hutner mineral salts solution (8), as well as the A-Z solution of Hoagland (33), inhibited the growth of *Pedomicrobium S-1290*.

Growth occurred in the presence of up to 0.1% (wt/vol) NaCl; media containing 1 to 5% NaCl allowed cells to survive but not to grow. Higher concentrations of NaCl were bactericidal.

Various buffers inhibited growth; 1 N acetic acid-NaOH (pH 5) and 0.1 M glycine-0.1 N NaOH (pH 9 or 10) prevented growth completely, and 67 mM Na₂HPO₄-KH₂PO₄ (pH 7) and 0.28 mM Na₂HPO₄-0.37 mM KH₂PO₄ (pH 9) allowed slow growth.

Table 5 shows the susceptibility of *Pedomicrobium S-1290* to various antibiotics. Intraperitoneal injection of 10⁸ cells in 1 ml of PSM did not cause any pathogenic reactions in guinea pigs within 3 weeks. Hemolysis was not observed on blood agar.

Pedomicrobium P-1196, Q-1197, R-1198, and T-1130. Strains P-1196, Q-1197, R-1198, and T-1130 were isolated from the same enrichment culture as strain S-1290. The morphologies of the cells and colonies of these strains were identical to those of S-1290. These strains also were gram negative and had poly-β-hydroxybutyric acid storage granules. In the presence of fulvic acid iron sesquioxide complexes or ethylenediaminetetraacetic acid-chelated Fe(II) and Mn(II), oxidized iron compounds were deposited on the cell surfaces, but manganese compounds were not. The DNA base compositions of these strains are shown in Table 2.

TABLE 4. *Growth of Pedomicrobium S-1290 with various nitrogen sources*^a

Nitrogen source	Growth ^b
Yeast extract (0.05%, wt/vol)	+
Peptone (0.05%, wt/vol)	+
Soytone (0.05%, wt/vol)	±
Vitamin-free Casamino Acids (0.05%, wt/vol)	±
2-Aminobenzoic acid (10 mM)	±
NaNO ₃ (10 mM)	±
Acetamide (10 mM)	±
Urea (10 mM)	±
<i>N</i> -acetylglucosamine (10 mM)	±
Methylamine hydrochloride (10 mM)	±
NaNO ₂ (0.01 M)	—
NaNO ₂ (0.58 M)	—
NaNO ₃ (1.06 M)	—

^a Growth after 9 days of incubation was calculated from the protein yield per milliliter of culture; protein yields were determined by the method of Lowry et al. (23).

^b +, Good growth (protein yield of >10 µg/ml); ±, poor growth (protein yield of 0.2 to 10 µg/ml); —, no growth.

FIG. 6. *P. ferrugineum* S-1290 pleomorphic cells after 10 days of incubation with a high concentration of organic substrate (1 g of ribose per liter, 5 g of peptone per liter, and 1 g of yeast extract per liter). Irregular cell shape, short hyphae, and voluminous, often free (arrows) granules of poly- β -hydroxybutyric acid indicate physiological abnormalities. Phase-contrast photographs of living cells fixed on an agar-coated slide. Bars = 10 μ m.

TABLE 5. Effects of antibiotics on growth of *Pedomicrobium* strains S-1290 and E-1129^a

Antibiotic	Effect on:	
	Strain S-1290	Strain E-1129
Ampicillin	++ ^b	—
Cephalothin	+	—
Bacitracin	—	+
Cycloserine	—	—
Polymyxin B	—	+
Nalidixic acid	—	—
Rifampin	++	—
Tetracycline	++	—
Gentamicin	+	++
Neomycin	+	++
Chloramphenicol	+	—
Nitrofurazon	+	—
Sulfanilamide	—	—

^a Determined by plate diffusion in autoclaved PSM. The central hole in each plate contained 0.4 ml of a solution containing 100 μ g of antibiotic per ml.

^b ++, Inhibition zone of >10 mm; +, inhibition zone of 1 to 10 mm; —, no inhibition zone.

All of these strains were catalase positive, and they grew well at 30°C under aerobic conditions. Slow and poor growth occurred with 0.1 to 1% fulvic acid iron sesquioxide complexes as sole carbon and nitrogen sources. Table 6 shows the utilization of some carbon sources by these strains. There were some minor differences compared with strain S-1290.

***Pedomicrobium* F-1225.** The cellular and colonial characteristics of strain F-1225 were also identical to those of strain S-1290, except for a

larger cell size (up to 1.7 by 2.5 μ m). Poly- β -hydroxybutyric acid storage granules were also observed. Growth on fulvic acid iron complexes as sole carbon and nitrogen sources was slow and poor and led to the accumulation of oxidized iron compounds on the cell surfaces.

***Pedomicrobium* E-1129.** With respect to most morphological properties, strain E-1129 was identical to the strains discussed above. The cells were spherical or oval and measured up to 0.9 by 1.5 μ m (Fig. 7 and 8). Up to five hyphae per cell were observed. Thin sections revealed intracytoplasmic membranes (Fig. 9), as well as poly- β -hydroxybutyric acid and polyphosphate. The colonial morphology of this strain corresponded to that of strain S-1290. However, colonies grown on fulvic acid iron complexes did not accumulate oxidized iron; instead, the negative Prussian blue reaction indicated a reduction of iron in a small zone around the colonies. In the presence of 0.75 mg of Mn(II) per liter (chelated with ethylenediaminetetraacetic acid), oxidized manganese compounds were deposited on the surfaces of spherical cells (11). Table 2 shows the DNA base composition of strain E-1129.

Strain E-1129 was catalase positive and grew well at 30°C under aerobic conditions. Slow and poor growth occurred with 0.1 to 1% fulvic acid iron sesquioxide complexes as sole carbon and nitrogen sources. Table 6 shows a selection of some of the carbon sources utilized by this strain. The sterile-filtered vitamin mixture (37) stimulated growth.

Table 5 shows the susceptibility of *Pedomi-*

TABLE 6. Growth of *Pedomicrobium* strains P-1196, Q-1197, R-1198, T-1130, and E-1129 with selected carbon sources^a

Strain	Carbon source (10 mM)					
	Glucose	Formate	Acetate	Caproate	Pyruvate	Lactate
P-1196	+	+	+	++	++	—
Q-1197	+	+	+	++	++	+
R-1198	—	—	++	++	++	—
T-1130	—	—	++	++	++	—
E-1129	++	++	++	++	++	++

^a Determined as increase in optical density at 650 nm after 6 days of incubation compared with the inoculum. ++, Increase in optical density of >0.02 U; +, increase in optical density of 0.005 to 0.2 U; —, no increase.

robium E-1129 to various antibiotics. Intraperitoneal injection of 10^8 cells suspended in 1 ml of PSM did not cause pathogenic reactions in guinea pigs within 3 weeks. There was no hemolysis on blood agar.

DISCUSSION

The original descriptions of the genus *Pedomicrobium* by Aristovskaya (1, 2) stressed the deposition of iron oxides or manganese oxides or both as a property which was useful in differentiating among the three species initially placed in the genus. Accordingly, six of the new isolates described here belong to the iron-depositing species, *P. ferrugineum*, whereas the one manganese-accumulating strain is identical to *P. manganicum*. Except for the type of heavy metal oxide accumulated, all of the new isolates described here have so many properties in common that they obviously have to be placed in the genus *Pedomicrobium*.

Along with heavy metal deposition, the morphological characteristics of the isolates investigated here corresponded in almost all respect with the original data (1, 3). Using my phase-contrast and electron micrographs supplemented with detailed descriptions, Aristovskaya herself kindly identified the new isolates as members of *P. ferrugineum* or *P. manganicum* (personal communication). Regarding physiology, only the manner of growth with humic extracts as the sole sources of nutrients could be compared, since no other information was available from the original descriptions; like the colonies in the cultures of Aristovskaya the colonies of the new strains described here "reached about 0.5 mm in diameter after several months" of incubation, and "in poured plates they developed primarily deep in the agar" (1). The ability to grow under microaerobic conditions was also demonstrated with the new isolates in an artificial atmosphere containing only 1% pO_2 .

Compared with the previous descriptions of *P. ferrugineum* and *P. manganicum* (1, 3–5), a few differences should be mentioned, although these may not be important. (i) Aristovskaya

described mother cells of *P. manganicum* that were $0.4 \mu m$ in diameter (1); under the conditions reported here, mother cells and mature swimmers of strain E-1129 measured up to 0.9 by $1.5 \mu m$. (ii) Aristovskaya (1) observed growth of *pedomicrobia* only on humic gel agar, whereas Khak-mun (18, 19) grew cultures in Beijerinck medium added to a humic gel. Khak-mun also obtained growth on a purely mineral medium supplemented with $MnCO_3$. In contrast, the isolates described here have been cultured with a large variety of inorganic and organic substrates. (iii) All reports concerning soil *pedomicrobia* (1, 4, 5, 18, 19) have mentioned high cell numbers of these organisms in the samples studied. This was not apparent in samples from various sites in northern Germany.

A variety of hyphal budding bacteria resembling *pedomicrobia* have been found by several workers. Unfortunately, these organisms do not fit the original descriptions of the three *Pedomicrobium* species completely, and they have not yet been studied in detail. Hirsch (13) isolated aquatic *Pedomicrobium*-like bacteria with the "ability to grow many hyphae from several sites on the rod-parts of the cells"; electron micrographs demonstrated up to 14 hyphae growing out of one cell (P. Hirsch, personal communication). Regarding the type of metal deposited on the cell surfaces, Ghiorse and Hirsch (12a) observed both iron and manganese accumulations on *Pedomicrobium*-like strains 868 and 869. Morphologically, these bacteria were closely related to *P. ferrugineum* but not to "*P. podsolicum*," which has been described as an organism which deposits both iron and manganese (2, 3). Manganese-accumulating *Pedomicrobium* strain E-1129 was also capable of depositing iron oxides when it was grown on media containing elemental iron (W. C. Ghiorse, personal communication). These observations suggest that iron deposition would be defined more correctly as iron deposition when organisms are grown on fulvic acid iron sesquioxide complexes. In this way, the original differentiation of *Pedomicrobium* species by Aristovskaya

FIG. 7. Morphology of typical *P. manganicum* E-1129 cell formations. Note different sites and numbers of hyphal outgrowths. Transmission electron microscope micrographs, Pt/C shadowed. Bar = 1 μ m.

remains valid, since that was the condition under which she observed iron deposition with *P. ferrugineum* but not with *P. manganicum*.

The difficulty of ascertaining the type of metal deposition can be further demonstrated with the hyphal budding bacterium T-37. This *Pedo-*

FIG. 8. Morphology of subsequent *P. manganicum* E-1129 cell generations. A, Mother cells with branched and unbranched hyphae; B, buds with lateral, broad hyphal bases; C, daughter cell exhibiting strain-specific broad hyphal base with long separation nose. Transmission electron microscope micrograph Pt/C shadowed. Bar = 1 μ m.

microbium-like strain, which was described originally as a manganese-depositing *Hyphomicrobium* sp. by Tyler and co-workers (6, 7, 39–43), has lost its ability to accumulate manganese oxides; instead, it now accumulates iron oxides (12). Thus, it follows that the type of metal oxide deposited cannot be the only criterion for the identification of the genus *Pedomicrobium* or for separating *Pedomicrobium* species. Additional properties should be considered.

Table 7 shows the differential characteristics of pedomicrobia and other genera of hyphal budding bacteria. The morphological and physiological properties listed demonstrate clearly the validity of the genus *Pedomicrobium*. Furthermore, still unpublished data and selected DNA-DNA homology studies (27) suggest the existence of forms that are intermediate between *Pedomicrobium* and *Hyphomicrobium*.

The *Pedomicrobium* isolates S-1290, P-1196, Q-1197, R-1198, and T-1130 clearly belong to *P. ferrugineum* of Aristovskaya. Although strain F-1225 accumulated iron when it was grown on humic gel agar, the data obtained so far are not yet sufficient to permit the classification of this strain; further investigations on this strain are in progress. Strain E-1129 certainly belongs to *P. manganicum*. At present, the minor differences between this strain and the corresponding *Pedomicrobium* species descriptions are regarded as unimportant.

None of the isolates described here fitted the descriptions of "*P. podsolicum*" (2, 3) and "*P. manganicum* subsp. *sachalinicum*" (18). Therefore, the original descriptions of both of these taxa should be maintained, although living cultures are not now known.

Based on the results discussed above, the fol-

FIG. 9. Thin section of a *P. manganicum* E-1129 cell exhibiting mesosome-like invaginations. Modified Kellenberger fixation, transmission electron microscope micrograph (W. C. Ghiorse). Bar = 0.5 μ m.

lowing emended descriptions are formally proposed for the genus *Pedomicrobium* and the species *P. ferrugineum* and *P. manganicum*.

Description of genus *Pedomicrobium* Aristovskaya 1961 emend. Gebers. (i) Morphology. Cells are spherical, oval, tetrahedral, or rod, pear, or bean shaped and measure 0.4 to 2.0 by 0.4 to 2.5 μ m. Up to five or more prosthecae (i.e., cellular outgrowths of constant diameter [hyphae]) are formed per cell; these are 0.15 to 0.3 μ m in diameter and vary in length with cultural conditions. At least one hypha originates laterally; less frequently, other hyphae can appear polarly or even subpolarly. True branching of the hyphae occurs. Multiplication is primarily by budding at the hyphal tips; mature buds either separate from the hyphae as unflagellated swimmers or remain attached. Buds may arise in an intercalary fashion by localized hyphal swelling. Occasionally, direct budding of mother cells or division of single mother cells has been observed (1, 3).

(ii) Colonial morphology. Two types of colony may develop on solid media. Type 1 colonies are round or convex and have even or radially frayed edges; they are yellowish red to dark brown in color, sometimes with concentric rings. These colonies have a soft consistency. Type 2 colonies are round, flat, and even; they

may be crateriform (i.e., grow downward into the agar), and the edges are even or radially frayed. Type 2 colonies are yellowish red to dark brown in color, and the cells in the center of the colonies are often lysed, giving a granular appearance to the colonies. These colonies have a cartilaginous consistency and may be removed from the agar intact.

Upon spreading on solid media, type 1 and 2 colonies give rise to both types of colonies. In the course of cultivation with periodic transfers to fresh medium, large colonies tend to predominate over small ones.

(iii) Cellular structure and composition.

In general, cells are gram-negative, although older cells may be gram-variable. Extracellular polymers stain with ruthenium red. Oxidized iron compounds or manganese compounds or both are deposited primarily on mother cells and also on hyphae. Intracytoplasmic membranes have been observed. Up to three granules of poly- β -hydroxybutyric acid are stored per cell. Small, dense granules stainable with Loeffler methylene blue suggest polyphosphates. Liquid cultures grown in the dark and with synthetic light may be yellowish white or reddish orange, respectively, due to pigments that have not been identified. The DNA base composition (two species, six strains) ranges from 64.5 to 66.0 mol%

TABLE 7. Differential characteristics of *Pedomicrobium* and some other hyphal budding bacteria

Taxon	No. of hyphae per mother cell	Origin of hyphae	Septation of hyphae	Diam of hyphae (μ m)	Flagellation of swarmer cells	Photosynthetic pigments	Growth with the following carbon sources: ^a		Growth with the following nitrogen sources: ^a		Growth in the presence of 5% NaCl ^a	Guanine-plus-cytosine content of DNA (mol%)	References
							Formate	Acetate	NaNO ₂ (4%)	NaNO ₃ (9%)			
<i>Pedomicrobium</i>	1-5 or more	Lateral and polar	Absent	0.15-0.3	Single, subpolar to polar	Absent	±	+	-	-	-	64.5-66.0	1-3, 11
<i>Hyphomicrobium</i>	1-2	Polar	Absent	0.3-0.4	Single, subpolar to polar	Absent	+	+	+	+	+	59.2-66.8	14, 24, 38
<i>Hyphomicrobium neptunium</i> ^b	1 or more	Polar	Absent	NT	1-2, polar or lateral	Absent	NT ^c	NT	NT	NT	NT	61.7	15, 22, 24
<i>Hyphomonas</i>	1	Polar	Absent	NT	Single, location not reported	Absent	-	-	NT	NT	NT	60.2-61.2	15, 24, 30
<i>Rhodomicrobium</i>	1 or 2	Polar, rarely lateral	Present	0.3	Peritrichous	Present	-	+	NT	NT	NT	61.8-63.8	9, 29

^a +, Good growth; ±, variable growth; -, no growth.^b *H. neptunium* is closely related to *Hyphomonas polymorpha* (14, 15).^c NT, Not tested.

guanine plus cytosine (T_m method).

(iv) **Physiological properties.** Cells are microaerophilic to aerobic, catalase positive, and heterotrophic. Slow poor growth occurs with 0.1 to 1% fulvic acid iron sesquioxide complexes as sole carbon and nitrogen sources; good growth occurs with 10 mM acetate, 10 mM caproate, or 10 mM pyruvate as the carbon source and 0.05% yeast extract, 0.05% peptone, 0.05% Casamino Acids, or 0.05% soytone as the nitrogen source. Vitamin mixtures stimulate growth; a lack of vitamins leads to pleomorphic cells. These organisms are not pathogenic for guinea pigs after intraperitoneal injection of 10^8 cells and are not hemolytic.

(v) **Occurrence.** Widely distributed in podzolic and other soils, freshwater lakes, iron springs, and seawater; probably ubiquitous.

Type species: *P. ferrugineum* Aristovskaya 1961.

Description of *P. ferrugineum* Aristovskaya 1961 emend. Gebers. Cells are spherical, oval, tetrahedral, or rod or bean shaped and measure 0.6 to 2 by 0.6 to 2.5 μ m. Multiplication is by budding at the tips of the hyphae, which are 0.2 μ m or less in diameter. Initially, buds are spherical to oval; they are motile by a single polar to subpolar flagellum. Deposition of oxidized iron compounds occurs when cells are grown with fulvic acid iron sesquioxide complexes, elemental iron powder, FeS, or iron paper clips; there is no accumulation of manganese compounds. Depositions occur primarily on mother cells and later on hyphae. The temperature for optimum growth is 30°C, and the temperature range is 10 to 40°C. The pH for optimum growth is 9.0; the pH range is 3.5 to 10.0. Good growth occurs with the following carbon sources (at 10 mM): acetate, caproate, succinate, fumarate, malate, gluconate, pyruvate, propanol, phenol, and glutamate. Slow and poor growth occurs with 10 mM oxalate, 10 mM lactate, 10 mM tartrate, 10 mM citrate, 10 mM methanol, 10 mM ethanol, 10 mM glycerol, 10 mM mannitol, 10 mM cholesterol, 0.3% paraffin, 10 mM aspartate, 10 mM tryptophane, or 10 mM alanine. And no growth occurs with 10 mM ribose, 10 mM glucose, 10 mM galactose, 10 mM fructose, 10 mM maltose, 10 mM lactose, 10 mM sucrose, 10 mM formate, 10 mM phenylalanine, 10 mM serine, or 10 mM lysine. Amino acids are utilized simultaneously as nitrogen sources.

Good growth occurs with 0.05% yeast extract or 0.05% peptone as a nitrogen source; slow and poor growth occurs with 0.05% soytone, 0.05% Casamino Acids, 10 mM 2-aminobenzoic acid, 10 mM NaNO₃, 10 mM acetamide, 10 mM urea, 10 mM *N*-acetylglucosamine, or 10 mM meth-

ylamine hydrochloride; and no growth occurs with 0.01 M NaNO₂, 0.58 M (4%) NaNO₂, or 1.06 M (9%) NaNO₃.

Growth occurs in the presence of up to 0.1% NaCl; no growth, but survival, has been observed in the presence of 1 to 5% NaCl. Higher concentrations of NaCl are bactericidal.

Cells are susceptible to the following antibiotics (at 100 µg/ml): ampicillin, rifampin, tetracycline, cephalothin, gentamicin, neomycin, chloramphenicol, and nitrofurazone. Cells are resistant to the following (at 100 µg/ml): bacitracin, cycloserine, polymyxin B, nalidixic acid, and sulfanilamide.

DNA base composition of the type strain: 65.3 ± 0.4 mol% guanine plus cytosine (*T_m* method). DNA base composition of four other strains: 65.3 ± 0.6 mol% guanine plus cytosine (*T_m* method).

All strains were isolated from podzolic soils in northern Germany (11).

Type strain: S-1290 (=ATCC 33119 =DSM 1540). Other strains: P-1196 (=ATCC 33116 =DSM 1541), Q-1197 (=ATCC 33117 =DSM 1542), R-1198 (=ATCC 33118 =DSM 1543), and T-1130 (=ATCC 33120 =DSM 1544).

Description of *P. manganicum* Aristovskaya 1961 emend. Gebers. Cells are spherical or oval to rod shaped and measure 0.4 to 0.9 by 0.4 to 1.5 µm. Multiplication is by budding at the hyphal tips.

Deposition of oxidized manganese compounds can occur in the presence of Mn(II). There is no accumulation of iron when cells are grown on fulvic acid iron sesquioxide complexes as sole iron sources.

Good growth occurs with the following sole carbon sources (at 10 mM): ribose, glucose, formate, acetate, caproate, pyruvate, and lactate.

Cells are susceptible to the following antibiotics (at 100 µg/ml): gentamicin, neomycin, bacitracin, and polymyxin B. Cells are resistant to the following antibiotics (at 100 µg/ml): ampicillin, cephalothin, cycloserine, nalidixic acid, rifampin, tetracycline, chloramphenicol, nitrofurazone, and sulfanilamide.

DNA base composition of the type strain: 64.7 ± 0.2 mol% guanine plus cytosine (*T_m* method).

Isolated from a quartzite rock pool, Forêt de Fontainebleau, France (11).

Type strain: E-1129 (=ATCC 33121 =DSM 1545).

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