Characterization of *Lawsonia intracellularis* gen. nov., sp. nov., the Obligately Intracellular Bacterium of Porcine Proliferative Enteropathy

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A novel obligately intracellular bacterium, ileal symbiont intracellularis, which was obtained from the intestines of pigs with proliferative enteropathy disease, was grown in pure cocultures with tissue cultures of rat cells. An examination of the 16S ribosomal DNA gene sequence revealed that the isolates which we obtained are members of the delta subdivision of the *Proteobacteria* and that the sequences of these organisms exhibit a level of similarity of 91% with the sequence of *Desulfovibrio desulfuricans* ATCC 27774. These isolates were homogeneous and differed in cellular morphology, acid fastness, phenotype, electrophoretic protein profile, and habitat from *Desulfovibrio* species. On the basis of the results of an integrated study of the phenotype and genotype of a consistent morphological entity found in particular porcine cells and associated with a well-defined clinical condition, we concluded that these bacteria belong to a previously undescribed genus and species, for which we propose the name *Lawsonia intracellularis* gen. nov., sp. nov. A species-specific recombinant DNA probe was cloned previously, and this probe was used to identify the bacterium in tissue culture cells and in the ileal epithelia of pigs with proliferative enteropathy disease. Coculture of the organism with a rat enterocyte cell line allowed us to designate strain NCTC 12656 the type strain and to describe the new genus and species. The organism which we cultured is pathogenic for pigs and causes proliferative enteropathy lesions in their ilea and colons, and Koch's postulates were fulfilled for this organism.

The recently described organism ileal symbiont (IS) intracellularis (5) was given a vernacular name until its taxonomic position could be clarified. Classification of IS intracellularis has been problematic because this organism is an obligately intracellular bacterium, which has been cultured only in established rat enterocyte cell cultures (11). Taxonomic studies of the organism before cultured organisms were available were performed with bacteria purified directly from infected tissues (5, 6, 12). It was found that this intracellular bacterium had novel immunological and DNA probe reactions (6, 12, 13) compared with those of *Campylobacter* species.

A subsequent taxonomic study of IS intracellularis in which the 16S ribosomal DNA (rDNA) was amplified and sequenced clearly revealed that the purified intracellular bacteria were homogeneous and belonged to the delta subdivision of the class *Proteobacteria* (5). The DNA sequences determined for each purified bacterial preparation were most similar to the sequences of a sulfate-reducing proteobacterium, *Desulfovibrio desulfuricans* (level of sequence similarity, 91%). There is considerable interest in the taxonomic position of IS intracellularis because of the etiologic role of this organism in proliferative enteropathy, a major disease that affects the economics of pig industries worldwide (14, 19).

The natural habitat of IS intracellularis is lying not bound by membranes within the cytoplasm of mammalian enterocytes, particularly enterocytes of pigs and hamsters (18, 19). Cell-free culture has not been attained. Enterocyte cell cultures are necessary for in vitro cultivation and maintenance of individual strains originally obtained from pig intestines (11). The results of recent experiments in which cultured bacteria were used as

oral inocula for pigs clearly established that IS intracellularis infection of pig intestines is the initiating factor for the development of the distinctive lesions of proliferative enteropathy (14). It is assumed that most natural infections occur as a result of animals consuming feed or other environmental material contaminated by infected feces.

The results of comparisons of intracellular bacteria (including IS intracellularis) obtained from proliferative enteropathy lesions in a variety of host species, including pigs, ferrets, foxes, rats, horses, and hamsters, in which immunoassays and specific antibodies were used indicated that there may be a group of related bacteria (19). Further analysis of the 16S rDNAs of the intracellular bacteria present in proliferative enteropathies in ferrets and hamsters revealed levels of similarity greater than 99% between the sequences of these organisms and the previously published IS intracellularis sequence determined by using bacteria obtained from pigs (5a). Therefore, it is possible that a new genus may contain several species that differ in their relationships to a diverse group of host species.

The major aim of this study was to determine the important taxonomic features of IS intracellularis grown in cell cultures, which allowed us to clearly delineate a new genus and propose a formal name. The habitat of this organism suggested that it should be compared with other intracellular bacteria, and the results of previous genomic studies suggested that IS intracellularis should be compared with *Desulfovibrio desulfuricans*. Difficulties in combining these two aims arose because of the difficulty of using obligately intracellular bacteria in many fermentative and biochemical taxonomic tests. Although *Rickettsia* spp. are the only obligately intracellular bacteria known to have a similar intracellular location (they occur free in the cytoplasm of epithelial cells) (15), any relationship with these bacteria was clearly ruled out by the DNA sequence data

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TABLE 1. Bacterial strains used in this study

Taxon	Strain	Source	Lesions ^a
IS intracellularis	NCTC 12656 ^T (= 1482/89 ^T)	Porcine	PHE
	NCTC 12657 (= 916/91)	Porcine	PHE
	1268/84	Porcine	PHE
	189/5/83	Porcine	PHE
	51/89	Porcine	PIA
	963/93	Porcine	PIA
<i>Desulfovibrio desulfuricans</i>	ATCC 29577 ^T	Freshwater	
	ATCC 27774	Freshwater	

^a PHE, proliferative hemorrhagic enteropathy; PIA, porcine intestinal adenomatosis.

(*Rickettsia* spp. are members of the alpha subdivision of the *Proteobacteria*) and by morphological and habitat differences (22) (*Rickettsia* spp. are generally small bacilli which require an insect host during the life cycle). Therefore, we decided to concentrate on relationships to *Desulfovibrio* spp. in our taxonomic comparisons because some *Desulfovibrio* species are morphologically similar to IS intracellularis (17) and can inhabit mammalian guts (7, 23) and one species (*Desulfovibrio desulfuricans*) exhibits the levels of DNA similarity mentioned above. Dissimilarity between the genus *Desulfovibrio* and IS intracellularis would therefore indicate that the latter organism should be placed in a novel genus rather than a new species.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. IS intracellularis strains were isolated in pure cultures in rat enterocyte cell line IEC-18 as described elsewhere (11). *Desulfovibrio* spp. were cultured on soft desulfovibrio agar in an anaerobic incubator as described elsewhere (23), as well as in several broth formulations, modified Barr's medium, cooked meat medium, and modified Starkey's medium (17, 23).

Phenotypic characterization. The phenotypic characteristics of the isolates were determined by preparing cell-free pellets for morphological observation by light microscopy and electron microscopy. We also attempted to grow the organisms in cell-free cultures with various media and atmospheres known to support the growth of *Desulfovibrio* spp. Our initial observations on the morphology and lack of growth of IS intracellularis under standard anaerobic growth conditions have been described elsewhere (5). The test to determine reduction of sulfate to hydrogen sulfide in lactate medium was performed by using standard methods (17). Briefly, viable inocula of each strain (approximately 10⁶ organisms) were added to 10-ml portions of lactate medium, and preparations were incubated at both 30 and 37°C for 2 days. We assumed that blackening of the medium indicated production of hydrogen sulfide. Uninfected IEC-18 cells were also tested.

Protein electrophoresis. Protein samples (sonicated whole cells and Sarkosyl-soluble fractions) were prepared and used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (12). The silver-stained protein band patterns on the gels were scanned and analyzed with an image analysis system (Bio-Image system; Millipore, Watford, United Kingdom), and similarity indices were computed.

Nucleic acid techniques. Total DNAs were purified from IS intracellularis strains 916/91 and 1482/89^T and (T = type strain) and both *Desulfovibrio desulfuricans* strains studied by using methods described elsewhere (6). The PCR was used to amplify eubacterial 16S rRNA genes from each IS intracellularis nucleic acid sample with three primer pairs (fD1 and rP1, fD1 and rP2, and fD2 and rP1) as described elsewhere (21). The amplification products were sequenced directly by annealing 500 ng of each product to oligonucleotide sequencing primers complementary to highly conserved regions of eubacterial 16S rDNA and by dideoxynucleotide chain termination sequencing with a Sequenase kit (U.S. Biochemicals, Cleveland, Ohio).

Phylogenetic analysis. The full-length 16S rDNA sequence determined for the genomic clone designated pCLO28 (5) was used for the phylogenetic analysis; this sequence provided more complete sequence data than the sequences obtained from clones generated by PCR. This sequence was manually aligned with rRNA sequences available from the GenBank data library. Some of these sequences were obtained in aligned form from David A. Stahl (University of Illinois) and Richard Devereux (Environmental Protection Agency, Gulf Breeze, Fla.). Our phylogenetic analyses were restricted to nucleotide positions which could be unambiguously aligned in all sequences. Least-squares distance matrix

FIG. 1. Scanning electron micrograph of *L. intracellularis* after release from cells in an enterocyte culture. The cells vary in length from 1.25 to 1.75 μ m and are slightly curved. No flagella are evident. Bar = 1 μ m.

analyses were performed by using the algorithm of DeSoete (1) with a correction for multiple unobserved mutations (16). Maximum-parsimony trees were constructed by using PAUP (version 3.1.1) (D. L. Swofford), while maximum-likelihood analyses were performed by using fast DNA-ML (version 1.0) distributed by the Ribosomal Database Project (10). Confidence estimates for the tree topologies generated by the parsimony and maximum-likelihood analyses were determined by bootstrap methods (4). Taxon addition order was randomized in the parsimony and maximum-likelihood analyses in order to remove bias introduced by order of sequence addition.

DNA probe analysis. A hypervariable region in the 16S rDNA sequence unique to IS intracellularis was identified, and a complementary oligonucleotide probe was synthesized and end labeled with digoxigenin as described previously (5). Primers fD1 and rP1 and an oligonucleotide probe prepared from the homologous region of *Campylobacter jejuni* were also labeled and used as controls; 10-fold dilutions of the DNA preparations were immobilized on nylon membranes and hybridized with these probes. Hybridization was detected by using an antidigoxigenin antibody according to the instructions of the manufacturer (Boehringer Mannheim, Lewes, East Sussex, United Kingdom).

Nucleotide sequence accession number. The 16S rDNA sequence of strain 1482/89^T has been deposited in the GenBank data library under accession number U30147.

RESULTS

Phenotypic characterization. IS intracellularis isolates were gram-negative bacilli whose cells were variably curved (occasionally sigmoid) and had tapering ends. The cells were 1.25 to 1.75 μ m long by 0.5 to 1.5 μ m wide. Flagella, pili, or fimbriae were not observed. Motility was not observed. Typical cells are shown in Fig. 1. These bacteria were acid fast when they were stained by the modified Ziehl-Neelsen method (19). Pelleted bacteria were pale tan. IS intracellularis isolates grew in cell cultures in the presence of ambient oxygen tensions of 5 to 15%, and optimum growth occurred in the presence of 8% oxygen in cultures in which the medium was 2 to 5 mm deep. Bacteria known to be viable when they infected cell cultures failed to grow on any cell-free medium under aerobic, microaerophilic, or anaerobic conditions at a variety of temperatures. We tested more than 80 media considered useful for growing *Desulfovibrio* species, including boosted complex media containing a variety of substrates, such as lactate, acetate, and propionate.

The *Desulfovibrio desulfuricans* isolates were gram-negative bacilli whose cells were variably curved and 2.5 to 10 μ m long by 0.5 to 1.5 μ m wide. *Desulfovibrio desulfuricans* and all other *Desulfovibrio* species have single polar flagella. Pelleted *Desul-*

FIG. 2. Diagram of the electrophoretic protein profiles of IS intracellularis strains and a *Desulfovibrio desulfuricans* strain. Whole cells were washed in 0.1 M Tris-buffered saline (pH 7.0), sonicated, mixed with SDS-PAGE sample buffer, and electrophoresed in 12.5% polyacrylamide homogenous gels. The proteins were stained by a silver staining method according to the instructions of the manufacturer (Phast-Gel; Pharmacia, Ltd., Uppsala, Sweden). The major bands were identified with an image analysis system (Bio-Image system; Millipore). Tracks 1, IS intracellularis strain 51/89; track 2, IS intracellularis strain 963/93; track 3, IS intracellularis strain 189/5/83; track 4, IS intracellularis strain 1268/84; track 5, IS intracellularis strain NCTC 12657; track 6, IS intracellularis strain NCTC 12656^T; track 7, *Desulfovibrio desulfuricans* ATCC 27774.

fovibrio species are black and are not stained by the modified Ziehl-Neelsen stain. Blackening of lactate medium was clearly visible in tubes inoculated with *Desulfovibrio* spp. strains, but blackening was not observed in any medium inoculated with IS intracellularis. Thus, there is no evidence that links IS intracellularis physiologically with *Desulfovibrio* spp.

Protein electrophoresis. One-dimensional SDS-PAGE of IS intracellularis whole-cell protein extracts produced 25 to 27 discrete bands. Figure 2 is a diagram of the whole-cell protein electrophoresis band patterns obtained. For each of the six IS intracellularis strains tested, major protein bands were observed at molecular weights of 53,000, 42,000, 37,000, and 30,000, and Sarkosyl-soluble protein bands were observed at molecular weights of 53,000, 47,000, and 42,000. A comparison of the bands of the six isolates revealed that at least 22 of the bands, including all of the major bands, were always present. This indicated that the six strains tested were members of a single phenon. The IS intracellularis patterns could be clearly distinguished from the patterns obtained for both *Desulfovibrio* spp. strains examined. A comparison of the bands obtained for IS intracellularis and *Desulfovibrio desulfuricans* revealed that only six of the *Desulfovibrio desulfuricans* bands corresponded to IS intracellularis bands at a confidence level of 95%.

FIG. 3. Phylogenetic tree based on 16S rRNA sequence data for sulfate-reducing bacteria and their relatives, showing the close relationship between *L. intracellularis* and members of the *Desulfovibrionaceae*. The tree was inferred from the results of a maximum-likelihood analysis of 1,047 homologous sequence positions for each organism. Bootstrap values that are more than 50% (100 replicates were examined) are indicated for maximum-likelihood and maximum-parsimony analyses, as described in the text. Bar = 10 mutations per 100 nucleotide sequence positions.

Genetic relationships. In unrestricted pairwise comparisons, the IS intracellularis rDNA sequences obtained by PCR from cultured strains 916/91 and 1482/89^T exhibited levels of similarity of 99.9% with each other and 99.7% with the sequence of genomic clone pCLO28 (5), which was obtained from DNA purified from tissues infected with IS intracellularis. A comparison of the IS intracellularis rDNA sequence with partial sequences of *Desulfovibrio multispirans* DSM 1743 and *Desulfovibrio* sp. strains Essex 6 and ATCC 7757 (3) revealed that IS intracellularis is not identical to any of these strains; however, the lengths of the strain DSM 1743, Essex 6, and ATCC 7757 sequences (147 to 207 nucleotides) prohibited more precise resolution of the phylogenetic relationships. Figure 3 shows a phylogenetic tree inferred from the results of a maximum-likelihood analysis of more extensive sequence data (1,047 nucleotide positions per sequence) for other sulfate-reducing bacteria and their relatives. Close relationships between the IS intracellularis sequence and the sequences of *Desulfovibrio desulfuricans* ATCC 27774, *Desulfovibrio vulgaris*, *Desulfovibrio* sp. strain PT-2, *Desulfovibrio longreachii*, and *Desulfomonas pigra* are supported by the results of bootstrap analyses in which both maximum-likelihood and parsimony methods were used (82 and 78 of 100 trees, respectively). A matrix of rRNA sequence similarity values is shown in Table 2.

We observed positive binding reactions between membrane-bound DNA derived from cultured IS intracellularis and a specific 16S rDNA-directed oligonucleotide DNA probe for IS intracellularis, as well as oligonucleotide primers fD1 and rP1. No reactions were observed with uninfected cells or *Desulfovibrio* spp. or between the *C. jejuni* oligonucleotide probe and IS intracellularis (Fig. 4).

DISCUSSION

In this paper we provide several major lines of evidence that IS intracellularis found in pigs belongs to a new genus and species, for which we propose the name *Lawsonia intracellularis*. The bacteriological characteristics, 16S rDNA sequence, and protein electrophoresis pattern of this organism clearly

TABLE 2. Levels of 16S rRNA similarity for *L. intracellularis* and other proteobacteria

Organism	% 16S rRNA similarity ^a															
	<i>Lawsonia intracellularis</i> ^b	<i>Desulfovibrio desulfuricans</i> ATCC 27774 ^b	<i>Desulfovibrio</i> sp. strain PT-2 ^b	<i>Desulfovibrio longreachii</i> ^b	<i>Desulfomonas pigra</i> ^b	<i>Desulfovibrio vulgaris</i> ^b	<i>Desulfovibrio baculatus</i> ^b	<i>Desulfovibrio salexigens</i> ^b	<i>Desulfovibrio desulfuricans</i> Norway 4 ^b	<i>Desulfovibrio gigas</i> ^b	<i>Desulfovibrio africanus</i> ^b	<i>Desulfovibrio desulfuricans</i> El Aghella Z ^b	<i>Desulfohalobacterium autotrophicum</i>	<i>Desulfococcus multivorans</i>	<i>Myxococcus xanthus</i>	<i>Escherichia coli</i>
<i>Desulfovibrio desulfuricans</i> ATCC 27774	91.4															
<i>Desulfovibrio</i> sp. strain PT-2	90.8	92.8														
<i>Desulfovibrio longreachii</i>	90.5	92.8	97.7													
<i>Desulfomonas pigra</i>	89.7	95.0	91.8	91.2												
<i>Desulfovibrio vulgaris</i>	89.6	91.8	94.3	93.6	91.2											
<i>Desulfovibrio baculatus</i>	86.8	89.7	88.6	88.3	88.7	88.1										
<i>Desulfovibrio salexigens</i>	86.6	88.2	87.8	88.7	87.4	89.0	87.5									
<i>Desulfovibrio desulfuricans</i> Norway 4	86.4	90.0	88.5	88.9	89.2	87.9	98.6	87.9								
<i>Desulfovibrio gigas</i>	86.4	89.5	89.6	89.8	89.4	88.0	87.8	87.0	88.2							
<i>Desulfovibrio africanus</i>	85.0	88.3	87.3	87.4	87.1	88.5	87.6	87.8	87.7	87.4						
<i>Desulfovibrio desulfuricans</i> El Agheila Z	84.1	88.2	86.8	86.1	86.9	88.0	88.3	90.4	88.8	87.5	88.5					
<i>Desulfohalobacterium autotrophicum</i>	85.0	84.5	83.8	83.2	84.0	86.5	82.8	86.7	83.7	82.3	83.3					
<i>Desulfococcus multivorans</i>	84.3	84.5	83.4	83.2	82.9	83.7	85.2	82.7	85.5	82.7	92.3	83.4	89.3			
<i>Myxococcus xanthus</i>	83.1	83.5	82.1	82.1	81.6	81.1	83.2	82.0	83.5	82.3	82.4	80.8	84.4	84.2		
<i>Escherichia coli</i>	82.7	82.1	81.4	81.5	80.7	81.1	82.1	80.4	81.2	81.0	81.4	82.6	82.4			
<i>Campylobacter jejuni</i>	78.7	78.9	78.9	77.3	78.4	78.1	78.1	77.7	78.5	77.7	77.8	77.5	79.3	78.8	78.2	78.7

^a Values were determined by comparing approximately 1,047 nucleotides in each sequence.

^b Member of the family *Desulfovibrionaceae* (3).

distinguish it from members of other genera. In particular, *L. intracellularis* can be distinguished from *Desulfovibrio desulfuricans* by its 16S rDNA sequence and specific DNA probe reactions. We were previously able to clone a recombinant DNA probe specific for pig-derived IS *intracellularis* (6).

The morphology and physiology of *L. intracellularis* in cell cultures and in tissues are distinct from the morphology and physiology of all previously described *Desulfovibrio* species. The latter are larger, possess flagella, are motile, and are richly pigmented with iron pigments, and no *Desulfovibrio* intracellular forms have been described. An analysis of the electrophoretic protein band patterns revealed that the IS *intracellularis* strains belong to a homogeneous cluster that is clearly distinct from *Desulfovibrio* spp. The patterns which we obtained for *L. intracellularis* are very similar to those reported previously for intracellular organisms thought to be *Campylobacter*-like organisms purified directly from tissues (12). In

the study of McOrist et al. (12), major outer membrane proteins were detected at molecular weights of 55,000 and 43,000, and these proteins corresponded to the 53,000- and 42,000-molecular-weight proteins observed in cultured *L. intracellularis*. The results of this study give a much clearer definition of the *L. intracellularis* proteins because of the improved yield and purity obtained with cultured organisms and because of new image analysis techniques. These proteins are clearly similar in various strains of *L. intracellularis*, indicating that the similarity in antigen-antibody reactions between strains (12, 13) and DNA sequences is not just an attribute of one similar major antigenic protein, such as a surface layer, but reflects a more fundamental similarity.

The life cycle of *L. intracellularis* in cell cultures and in tissues clearly distinguishes it from other obligately intracellular bacteria. For example, *Chlamydia* spp. remain inside membranous compartments in cells following entry into the cells (15). Only some *Rickettsia* species are similar to *L. intracellularis* in that they are able to live and multiply free in the cytoplasm of epithelial cells. However, *Rickettsia* species are members of the alpha subdivision of the 16S rDNA sequence groups, are not known to invade mammalian enterocytes, and are generally smaller than *L. intracellularis* (21, 22). *L. intracellularis* is clearly an oxygen-tolerant obligately intracellular bacterium, as it did not grow at all on any conventional media or even in chicken egg membranes (5, 20) yet did grow in rat enterocyte cell cultures (11). Given the technical difficulties in culturing obligately intracellular bacteria, *L. intracellularis* in biological samples could be identified more easily by performing DNA-DNA hybridization experiments with specific genomic DNA probes, as described elsewhere (6). PCR techniques with specific primers have been developed recently (9). We anticipate that these techniques will be suitable for detect-

FIG. 4. Dot blot reactions of 10-fold dilutions of bacterial cells hybridized with digoxigenin-labeled primers fD1 and rP1 (a), the IS *intracellularis* 16S rDNA sequence oligonucleotide probe (b), or the *C. jejuni* 16S rDNA-specific oligonucleotide probe (c). Lanes 1, DNA purified from IS *intracellularis* grown in cell culture; lanes 2, DNA purified from *Desulfovibrio desulfuricans* ATCC 29577^T; lanes 3, DNA purified from a cell culture.

ing organisms in fecal samples and for other applications in clinical and epidemiological studies.

While our analysis of the 16S rDNA gene sequences revealed a level of homology of 91% with *Desulfovibrio desulfuricans*, the other characteristics of *Desulfovibrio desulfuricans* clearly indicate that this species is substantially different from *L. intracellularis*. Even if intracellular forms of *Desulfovibrio* species were found, there would still be enough genetic, phenotypic, and protein differences to warrant the new genus proposed in this paper. In particular, anaerobic growth and sulfate reduction, which are widely used as characteristics of *Desulfovibrio* spp. and may be beneficial in the marine habitat of these organisms (23), are not likely to be preferred enzymatic pathways for intracellular bacteria, as hydrogen sulfide is likely to be toxic to the supporting host cells and the dissolved oxygen tension in the porcine ileum is 5 to 10% (8), the preferred range for *L. intracellularis*. However, *Desulfovibrio* spp. are common inhabitants of intestines of mammals, including humans (7). Therefore, *L. intracellularis* may represent an evolutionary move away from anaerobic sulfate reduction. Whether other members of the *Desulfovibrionaceae* have the abilities to enter cells and survive without needing to reduce sulfates is an interesting question that will require investigation of mutant or degenerate strains.

The protein profiles of *Desulfovibrio* spp. have not been published previously, even though there are considerable data on individual enzyme proteins (2, 3). Because of the wide range of G+C contents found in members of the *Desulfovibrionaceae* (46 to 61 mol%) and the necessity and suitability of 16S rDNA gene sequencing for clarifying phylogenetic relationships (3), we used 16S rDNA gene sequencing along with DNA probe analysis and protein electrophoresis profiling for detailed comparisons.

The type strain of *L. intracellularis* has been designated previously, and a partial formal description of *L. intracellularis* has been published previously (5); a more complete description is given below.

Description of *Lawsonia intracellularis* gen. nov., sp. nov. *Lawsonia intracellularis* (Law.soi.ia. L.n. *Lawsonia*, in honor of G. H. K. Lawson, the discoverer of the bacterium; in.tra.cell.u. la'ris. L. adv. *intra*, within; M. L. n. *cellula* cell; L. adj. *intracellularis* within small cells, referring to the presence of the organism within enterocytes).

Non-spore-forming curved rods (ca. 1.5 by 0.35 μ m). Gram-negative cells retain carbol-fushin when they are stained by the modified Ziehl-Neelsen method. Nonflagellated. Nonpigmented. Sections reveal the cell wall profile of a gram-negative bacterium and the protoplasmic structure of a procaryote. Cells characteristically replicate within the cytoplasm, are not enclosed by membrane-bound vacuoles, and occur in epithelial cells in the ilea of pigs. Best revealed in histological sections by silver-staining techniques. Intracellular organisms exhibit acid-fast staining in smears of intestinal epithelium and enterocytes with the modified Ziehl-Neelsen acid-fast stain. Multiplies by septum formation. Does not reduce sulfate. Cannot be cultivated in cell-free media. Grows intracellularly in enterocytes and requires a microaerophilic atmosphere for growth. The sequence of 16S rDNA shows that these bacteria are members of the delta subdivision of the *Proteobacteria*. Distinct from certain *Desulfovibrio* spp. in electrophoretic protein profiles, DNA probe reactions, and monoclonal antibody reactions. Isolated from the intestinal epithelial cells of pigs with proliferative enteropathies. Pathogenic for pigs with an intestinal flora. The type strain is strain NCTC 12656 (= 1482/89).

Designation of the type strain. The type strain is 1482/89, which was cocultured in a rat enterocyte cell line that was

infected with a suspension of bacteria obtained from a pig with proliferative enteropathy and maintained in a rat enterocyte cell culture. This strain reproduces effectively in rat enterocytes without noticeable cytopathic effects or threat to culture survival. Strain 1482/89^T has been deposited in the National Collection of Type Cultures, Colindale, London, United Kingdom, as strain NCTC 12656^T. The bacterium growing in a cell culture conforms to the genus and species description given above.

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REFERENCES

1. DeSoete, G. 1983. A least squares algorithm for fitting additive trees to proximity data. *Psychometrika* **48**:621-626.
2. Devereux, R. M., M. Delaney, F. Widdel, and D. A. Stahl. 1989. Natural relationships among sulfate-reducing eubacteria. *J. Bacteriol.* **171**:6689-6695.
3. Devereux, R. M., S. H. He, C. L. Doyle, S. Orkland, D. A. Stahl, J. LeGall, and W. B. Whitman. 1990. Diversity and origin of *Desulfovibrio* species: phylogenetic definition of a family. *J. Bacteriol.* **172**:3609-3619.
4. Felsenstein, J. 1985. Confidence limits on phylogenies: an approach utilizing the bootstrap. *Evolution* **39**:783-791.
5. Gebhart, C. J., S. M. Barnes, S. McOrist, G. F. Lin, and G. H. K. Lawson. 1993. Ileal symbiont intracellularis, an obligate intracellular bacterium of porcine intestines showing a relationship to *Desulfovibrio* species. *Int. J. Syst. Bacteriol.* **43**:533-538.
- 5a. Gebhart, C. J., and F. E. Dewhirst. Unpublished data.
6. Gebhart, C. J., G. F. Lin, S. McOrist, G. H. K. Lawson, and M. P. Murtaugh. 1991. Cloned DNA probes specific for the intracellular *Campylobacter*-like organism of porcine proliferative enteritis. *J. Clin. Microbiol.* **29**:1011-1015.
7. Gibson, G. R., G. T. MacFarlane, and J. H. Cummings. 1993. Sulphate reducing bacteria and hydrogen metabolism in the human large intestine. *Gut* **34**:437-439.
8. Hillman, K. A., A. Smith, and W. Jones. 1993. Dissolved oxygen concentration in the porcine gastro-intestinal tract. *Lett. Appl. Microbiol.* **6**:8-11.
9. Jones, G. F., G. E. Ward, M. P. Murtaugh, G. Lin, and C. J. Gebhart. 1993. Enhanced detection of intracellular organism of swine proliferative enteritis, ileal symbiont intracellularis, in feces by polymerase chain reaction. *J. Clin. Microbiol.* **31**:2611-2615.
10. Larsen, N., G. J. Olsen, B. L. Maidak, M. J. McCaughey, R. Overbeek, T. J. Mackie, T. L. Marsh, and C. R. Woese. 1993. The Ribosomal Database Project. *Nucleic Acids Res.* **21**:3021-3023.
11. Lawson, G. H. K., S. McOrist, S. Jasni, and R. A. Mackie. 1993. Intracellular bacteria of porcine proliferative enteropathy: cultivation and maintenance in vitro. *J. Clin. Microbiol.* **31**:1136-1142.
12. McOrist, S., R. Boid, and G. H. K. Lawson. 1989. Antigenic analysis of *Campylobacter* species and an intracellular *Campylobacter*-like organism associated with porcine proliferative enteropathies. *Infect. Immun.* **57**:957-962.
13. McOrist, S., R. Boid, G. H. K. Lawson, and I. McConnell. 1987. Monoclonal antibodies to intracellular *Campylobacter*-like organisms of the porcine proliferative enteropathies. *Vet. Rec.* **121**:421-422.
14. McOrist, S., S. Jasni, R. A. Mackie, N. MacIntyre, N. Neef, and G. H. K. Lawson. 1993. Reproduction of porcine proliferative enteropathy with pure cultures of ileal symbiont intracellularis. *Infect. Immun.* **61**:4296-4292.
15. Moulder, J. W. 1985. Comparative biology of intracellular parasitism. *Microbiol. Rev.* **49**:298-337.
16. Olsen, G. J. 1987. Earlier phylogenetic branchings: comparing rRNA-based evolutionary trees inferred with various techniques. *Cold Spring Harbor Symp. Quant. Biol.* **52**:825-833.
17. Postgate, J. R. 1979. The sulfate-reducing bacteria. Cambridge University Press, Cambridge.
18. Rowland, A. C., and G. H. K. Lawson. 1974. Intestinal adenomatosis in the pig: immunofluorescent and electron microscopic studies. *Res. Vet. Sci.* **17**:323-330.
19. Rowland, A. C., and G. H. K. Lawson. 1992. Porcine proliferative enteropathies, p. 560-569. In A. D. Leman, B. E. Straw, W. L. Mengeling, S. D'Allaire, and D. J. Taylor (ed.), *Diseases of swine*, 7th ed. Iowa State University Press, Ames.

20. **Ward, G. E., K. J. Harp, and G. F. Jones.** 1991. Use of embryonating eggs for isolation of *Campylobacter* species from intestines of swine with proliferative enteritis. *Am. J. Vet. Res.* **52**:810–812.
21. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**:697–703.
22. **Weisburg, W. G., M. E. Dobson, J. E. Samuel, G. A. Dasch, L. P. Mallavia, O. L. Baca, L. Mandelco, J. E. Sechrest, E. Weiss, and C. R. Woese.** 1989. Phylogenetic diversity of the rickettsiae. *J. Bacteriol.* **171**:4202–4206.
23. **Widdel, F., and N. Pfennig.** 1984. Dissimilatory sulfate- or sulfur-reducing bacteria, p. 663–679. *In* N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. Williams & Wilkins, Baltimore.