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Phylogenetic evidence for the transfer of *Eubacterium lentum* to the genus *Eggerthella* as *Eggerthella lenta* gen. nov., comb. nov.

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Eubacterium lentum **has unique phenotypic characters within the genus** ***Eubacterium*****. The 16S rRNA sequence of** ***Eubacterium lentum*** **was determined and its phylogenetic position was defined. This micro-organism is a member of the genus** ***Eubacterium*** **but it is not closely related to** ***Eubacterium limosum*****, the type species of the genus** ***Eubacterium*****, and is nearer to** ***Collinsella aerofaciens*** **and** ***Coriobacterium glomerans*****. A PCR-based identification system using species-specific primers designed on the basis of DNA sequences encoding the 16S rRNA of strains of** ***Eubacterium lentum*****,** ***Collinsella aerofaciens*** **and** ***Coriobacterium glomerans*** **is described. A species-specific primer set can distinguish** ***Eubacterium lentum*** **from** ***Eubacterium limosum*** **or closely related species including** ***Collinsella aerofaciens*****,** ***Coriobacterium glomerans*** **and** ***Atopobium*** **species. This species-specific PCR method can be used to identify** ***Eubacterium lentum*****-like species isolated from human faeces. On the basis of the 16S rRNA sequence divergence from** ***Collinsella aerofaciens*** **and** ***Coriobacterium glomerans*** **and the presence of unique phenotypic characters, a new genus,** ***Eggerthella*** **gen. nov., is proposed for** ***Eubacterium lentum*****, with one species,** ***Eggerthella lenta*** **comb. nov. The type strain of** ***Eggerthella lenta*** **is JCM 9979^T.**

Keywords: *Eggerthella lenta*; 16S rDNA; PCR using genus-specific primers

INTRODUCTION

Eubacterium lentum Prévot's strain 1899B was isolated from a rectal tumour in 1938 (Prévot, 1938). Eggerth provided the original description of *Eubacterium lentum* on the basis of 23 strains from human faeces; the organism was originally named '*Bacteroides lentus*', but no type strain was designated (Eggerth, 1935). Moore *et al.* (1971) emended the description of *Eubacterium lentum* on the basis of a study of 53 strains, including another 38 strains isolated from human infections and three strains labelled *Eubacterium lentum* from the Prévot collection. *Eubacterium lentum* is a Gram-positive, obligately anaerobic, non-spore-forming rod. *Propionibacterium*, *Lactobacillus*, *Actinomyces* and *Bifidobacterium* have the same phenotype but these were differentiated by acid production from glucose. *Propionibacterium* species produce propionic acid as a major product, *Lactobacillus* species produce lactic acid as the sole major acid product, *Actinomyces* species produce large amounts of succinic acid (in the presence of CO_2) and lactic acid, sometimes with acetic and formic acids, and *Bifidobacterium* species produce acetic and lactic acids (acetic > lactic), with or without formic acid, as the major acid products. Species that are Gram-positive, obligately anaerobic, non-spore-forming rods and do not belong to any of the above genera are classified as *Eubacterium*. *Eubacterium lentum* produces little or no gas or product from 0 to 1·5 meq of acetic, formic, succinic or lactic acids, or from all four acids, so it is defined as a member of *Eubacterium*. Cells of *Eubacterium lentum* are small, non-motile rods that do not produce acid from glucose and do not produce indole or liquefy gelatin; these characters differentiate the taxon from other species of the genus *Eubacterium*.

The intestinal microflora in humans and animals consists of several micro-organisms, including

The DDBJ accession number for the 16S rRNA sequence of strain JCM 9979^T is AB011817.

anaerobes and aerobes. The genus *Eubacterium* of anaerobic, Gram-positive, non-spore-forming rods is one of the predominant micro-organisms in the intestinal microflora. *Collinsella aerofaciens*, which has been transferred from *Eubacterium* because of its phylogenetic and phenotypic characters (Kageyama *et al.*, 1999), *Eubacterium rectale*, *Eubacterium lentum* and *Eubacterium cylindroides* are dominant in the human intestine (Benno *et al.*, 1986; Finegold & Sutter, 1978; Moore & Holdeman, 1974). *Eubacterium lentum* has anti-tumour mechanisms and the bacterial components responsible for the anti-tumour effect have been investigated (Hatta, 1995; Morinaga *et al.*, 1988). *Eubacterium lentum* inactivates the cardiac glycoside digitoxin by reducing the double bond in the lactone ring (Chandrasekaran *et al.*, 1987).

The genus *Eubacterium* has a broad definition, so this genus has for a long time acted as a depository for a large number of phenotypically diverse species. It is not only phenotypically heterogeneous, but also phylogenetically heterogeneous, with species dispersed among many genera, and includes organisms that are not related phylogenetically.

In this study, in order to understand the taxonomic position of *Eubacterium lentum* JCM 9979^T, we determined the sequence of the 16S rRNA gene and analysed its phylogenetic position. On the basis of this 16S rRNA sequence data and the phenotypic characters, we propose that this organism should be classified as *Eggerthella lenta* gen. nov., comb. nov. A simple PCR identification method using *Eggerthella lenta* species-specific primers was established and its simplicity and utility were confirmed.

METHODS

Bacterial strains studied and cultivation. The bacterial strains used in this study were 17 strains that have *Eubacterium lentum*-like characters isolated from human faeces (Table 1) and the following type strains: *Eubacterium lentum* JCM 9979^T, *Collinsella aerofaciens* JCM 10188^T, *Coriobacterium glomerans* JCM 10262^T, *Eubacterium limosum* JCM 6421^T, *Eubacterium barkeri* JCM 1389^T, *Atopobium minutum* JCM 1118^T, *Eubacterium fossor* JCM 9981^T, *Eubacterium combesii* JCM 9988^T, *Eubacterium multiforme* JCM 6484^T, *Eubacterium nitritogenes* JCM 6485^T, *Eubacterium tenue* JCM 6486^T, *Eubacterium moniliforme* JCM 9990^T, *Eubacterium cylindroides* JCM 10261^T, *Lactobacillus plantarum* JCM 1149^T, *Bifidobacterium bifidum* JCM 1255^T and *Propionibacterium propionicum* JCM 5830^T. All bacterial strains were cultivated in an anaerobic chamber for 2 d at 37 °C on EG agar (premixed EG agar; Eiken Chemical Co.), which contains 3 g beef extract, 5 g yeast extract, 10 g peptone, 1·5 g glucose, 0·5 g L-cysteine HCl, 0·2 g L-cystine, 4 g Na_2HPO_4, 0·5 g soluble starch, 0·5 g Tween 80, 0·5 g silicone and 15 g agar l^{-1}, pH 7·7, supplemented with 5% horse blood.

16S rDNA sequencing. The 16S rRNA gene was amplified by PCR with prokaryotic 16S rDNA universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR was performed with a DNA thermal cycler (Perkin-Elmer Cetus) using 30 cycles consisting of denaturation at 94 °C for 60 s, primer annealing at 55 °C for 150 s and primer extension at 72 °C for 150 s (with 30 s added per cycle). Ligation was performed using plasmid pT7 Blue T vector (Novagen) and a ligation kit (Takara Shuzo) as recommended by the manufacturer. Transformation was done using *Escherichia coli* MJ1190 competent cells and colonies were selected for analysis. Plasmids were prepared for sequencing by the alkaline lysis method. Sequencing was performed using the ALFred AutoCycle Sequencing Kit (Pharmacia Biotech) with an ALFexpress DNA sequencer (Pharmacia Biotech). Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura & Ohta, 1972) and phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987). The topology of the trees was evaluated by performing a bootstrap analysis of the sequence data with CLUSTAL W software (Thompson *et al.*, 1994) (Fig. 1). The DNAML program in the PHYLIP 3.5c package (Felsenstein, 1985) was used for the maximum-likelihood analysis, with the default transition/transversion ratio of 2·0 (Fig. 2).

Table 1. List of *Eubacterium lentum*-like strains isolated from human faeces and their sources

Strain	Source	Year
A2-3	Patient with ulcerative colitis	1978
A2-184	Canadian	1982
A3-99	Healthy adult	1981
A3-104	Healthy adult	1981
A3-112	Healthy adult	1981
A3-125	Healthy adult	1981
A3-252	Healthy adult	1981
A5-166	Infant	1984
A5-189	Patient with ulcerative colitis	1982
A5-190	Patient with Crohn's disease	1982
A9-162	Patient with colon cancer	1979
RCA1-1	Healthy adult	1981
RCA1-16	Healthy adult	1981
RCA1-18	Healthy adult	1981
RCA2-10	Healthy adult	1981
RCA2-37	Healthy adult	1981
RCA58-92	Healthy adult	1981

Primer design. In order to understand the taxonomic position of *Eubacterium lentum*, we determined the 16S rRNA sequences of *Eubacterium lentum* JCM 9979^T and compared these sequences with other *Eubacterium* species and some other genera (obtained from the Ribosome Database Project). As a result of this comparison, we found that *Eubacterium lentum* was far from other *Eubacterium* species but near to *Collinsella aerofaciens* and *Coriobacterium glomerans*. Therefore, to design the species-specific primers, we compared the 16S rRNA sequences of *Eubacterium lentum*, three *Collinsella aerofaciens* strains and *Coriobacterium glomerans* and searched for positions that differed between *Eubacterium lentum*, *Collinsella aerofaciens* and *Coriobacterium glomerans*. We designed the species-specific primers so that the G+C content was about 50 mol% and the number of bases was about 20. We designed two sets of primers: LEN-F1 and LEN-R1 and LEN-F2 and LEN-R2. The closest known relatives of these

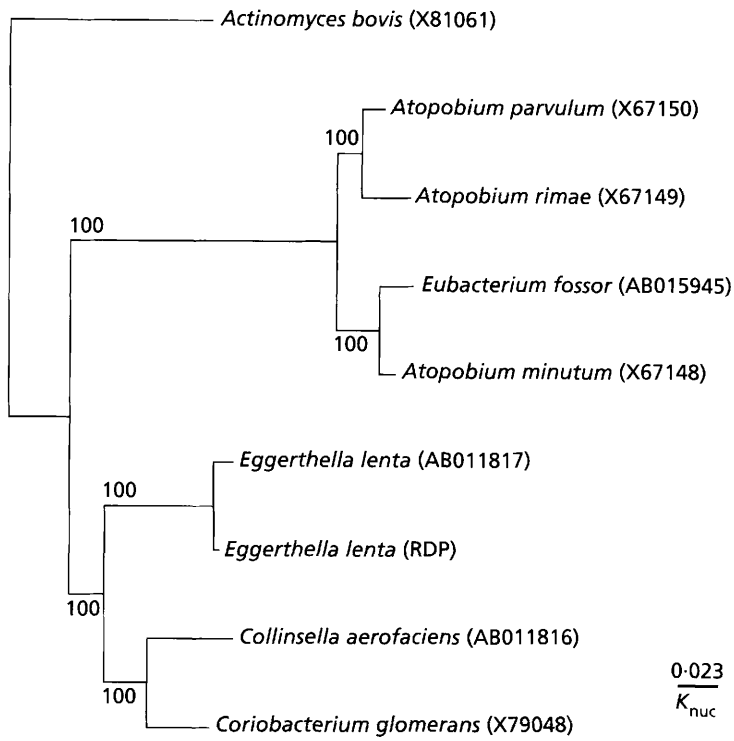

Fig. 1. Unrooted phylogenetic tree of *Eggerthella lenta* (= *Eubacterium lentum*) and close relatives derived from 16S rDNA sequences. The tree was created using the neighbour-joining method and K_{nuc} values. The numbers on the tree indicate bootstrap values for the branch-points. The sequence data for species other than *Eggerthella lenta* (AB011817) were obtained from the database.

primers were determined by performing a sequence database search by using FASTA.

PCR. PCR was performed with a DNA thermal cycler using 25 cycles consisting of denaturation at 94 °C for 60 s, primer annealing at 63 °C for 150 s and primer extension at 72 °C for 150 s using one of the sets of primers mentioned above. The sample DNA was prepared by suspending the cultured colony in distilled water, heating at 100 °C for 5 min and then cooling. Each amplification was performed using *Eubacterium lentum* as a positive control and *Collinsella aerofaciens* as a negative control. After PCR, an 8 µl aliquot of amplified sample from each PCR tube was electrophoresed through a 2% agarose gel (Sigma) in TAE buffer for 30 min at 100 V. Amplification products were visualized and photographed under a UV light transilluminator after 30 min of ethidium-bromide staining. The molecular masses of the amplicons were determined by comparison with commercial DNA molecular mass markers.

Primer species specificity. Primer specificity was defined as the ability of a primer to anneal specifically only to *Eubacterium lentum* 16S rRNA. The specificity of the primers was tested against the following organisms: *Eubacterium lentum* JCM 9979^T, *Eubacterium limosum* JCM 6421^T, *Eubacterium barkeri* JCM 1389^T, *Eubacterium fossor* JCM 9981^T, *Eubacterium combesii* JCM 9988^T, *Eubacterium multiforme* JCM 6484^T, *Eubacterium nitritogenes* JCM 6485^T, *Eubacterium tenue* JCM 6486^T, *Eubacterium moniliforme* JCM 9990^T, *Eubacterium cylindroides* JCM 10261^T and members of six other genera (*Collinsella aerofaciens* JCM 10188^T, *Coriobacterium glomerans* JCM 10262^T, *Atopobium minutum* JCM 1118^T, *Lactobacillus plantarum* JCM 1149^T, *Bifidobacterium bifidum* JCM 1255^T and *Propionibacterium propionicum* JCM 5830^T).

Amplification of isolated strains. First, strains were isolated from human faeces by culturing on EG agar plates under anaerobic conditions and many colonies were obtained. We then examined cell morphology and sugar fermentation to check whether these strains were *Eubacterium lentum*. Finally, 17 *Eubacterium lentum*-like strains isolated from human faeces were tested by this PCR method.

RESULTS

16S rDNA sequence analysis

More than 1400 bases of the 16S rDNA sequence (positions 28–1492; *Escherichia coli* numbering system) of *Eubacterium lentum* JCM 9979^T were determined and this sequence has been deposited in the DDBJ database. A database search demonstrated that *Eubacterium lentum* belongs to the family *Coriobacteriaceae*, which contains the genera *Coriobacterium* and *Atopobium*. A phylogenetic analysis was performed including all species of the family *Coriobacteriaceae* to elucidate the topology of *Eubacterium lentum* and the species of the family *Coriobacteriaceae* in two different analysis (a neighbour-joining method and maximum-likelihood analysis) (Figs 1 and 2). Both phylogenetic analyses showed that *Eubacterium lentum* JCM 9979^T is member of the *Coriobacteriaceae* and is closely related to the species *Collinsella aerofaciens* and *Coriobacterium glomerans* (Haas & König, 1988).

Primer design and specificity

The results of 16S rRNA sequence analysis revealed that *Eubacterium lentum* has high sequence identity to *Collinsella aerofaciens* and *Coriobacterium glomerans*.

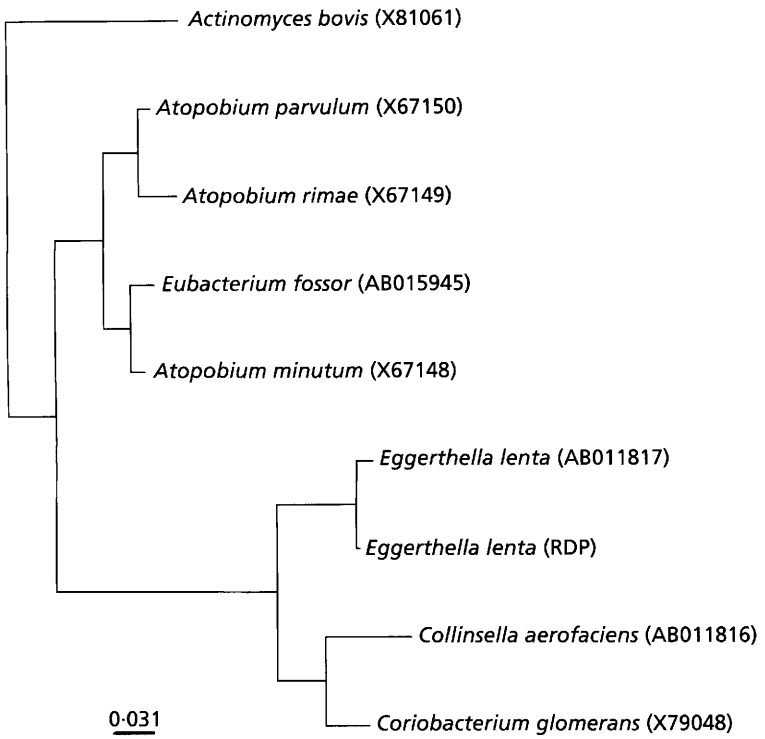

Fig. 2. Unrooted phylogenetic tree of *Eggerthella lenta* (= *Eubacterium lentum*) and close relatives derived from 16S rDNA sequences. The tree was created using maximum-likelihood analysis. The sequence data for species other than *Eggerthella lenta* (AB011817) were obtained from the database.

Table 2. Percentage identity of 16S rRNA of *Eubacterium lentum* to other related taxa

Taxon	Bases	1	2	3	4	5
1. *Eubacterium lentum*	1472	–				
2. *Collinsella aerofaciens*	1463	85·4	–			
3. *Coriobacterium glomerans*	1435	87·8	90·7	–		
4. *Atopobium minutum*	1492	85·7	85·0	85·8	–	
5. *Atopobium rimae*	1376	84·5	83·5	84·3	91·8	–
6. *Atopobium parvulum*	1396	85·1	84·7	84·9	93·4	96·1

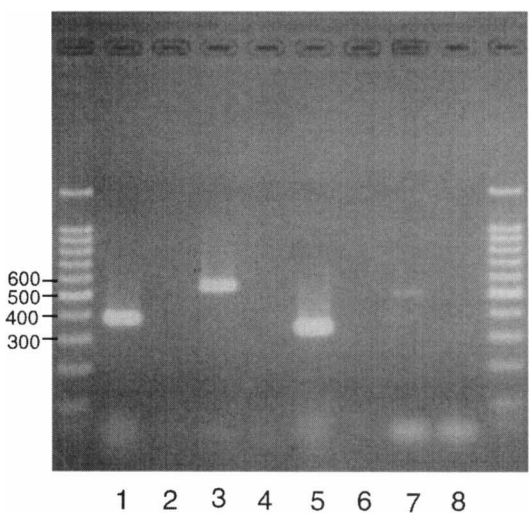

Fig. 3. Amplification of the 16S rRNA gene by PCR with primers LEN-F1, LEN-F2, LEN-R1 and LEN-R2. Lanes on both ends contain molecular mass markers. DNA from *Eggerthella lenta* (odd-numbered lanes) and *Collinsella aerofaciens* (even-numbered lanes) was amplified with primers LEN-F1 and LEN-R1 (lanes 1 and 2), LEN-F1 and LEN-R2 (lanes 3 and 4), LEN-F2 and LEN-R1 (lanes 5 and 6), and LEN-F2 and LEN-R2 (lanes 7 and 8). A product was always amplified from DNA of *Eggerthella lenta* JCM 9979^T and no product was amplified from DNA of *Collinsella aerofaciens* JCM 10188^T with any primer combination.

Overall 16S rRNA sequence identities are given for *Eubacterium lentum*, *Collinsella aerofaciens*, *Coriobacterium glomerans* and *Atopobium* species in Table 2. We next compared the 16S rRNA sequences of *Eubacterium lentum*, three *Collinsella aerofaciens* strains and *Coriobacterium glomerans* and found mismatched positions. Finally, we designed and synthesized two sets of primers: LEN-F1 (5′-CATGAAGTGGCGAACGGGTGA-3′; positions 94–116, *Escherichia coli* numbering system), LEN-F2 (5′-CTTGCTCCGGACAACCTTGGGA-3′; positions 139–160), LEN-R1 (5′-CTTCTGCTGCAGGTACCGTCGAATT-3′; positions 497–452) and LEN-R2 (5′-TCTACCGAACTCGAGCCTCCCA-3′; positions 665–644). All primers consisted of about 20 bases and their G+C content was about 50 mol%. We attempted PCR using four combinations of primers: LEN-F1+LEN-R1, LEN-F1+LEN-R2, LEN-F2+LEN-R1 and LEN-F2+LEN-R2. The amplicons expected contained 383 bp (positions 94–497) (LEN-F1+LEN-R1), 550 bp (positions 94–665) (LEN-F1+LEN-R2), 340 bp (positions

Table 3. Results of species-specific PCR

Results of PCR with various primers are shown as positive (+) or negative (−).

Taxon	Strain	Primers			
		LEN-F1+ LEN-R1	LEN-F1+ LEN-R2	LEN-F2+ LEN-R1	LEN-F2+ LEN-R2
Eubacterium lentum	JCM 9979^T	+	+	+	+
Collinsella aerofaciens	JCM 10188^T	−	−	−	−
Coriobacterium glomerans	JCM 10262^T	−	−	−	−
Eubacterium limosum	JCM 6421^T	−	−	−	−
Eubacterium barkeri	JCM 1389^T	−	−	−	−
Atopobium minutum	JCM 1118^T	−	−	−	−
Eubacterium fossor	JCM 9981^T	−	−	−	−
Eubacterium combesii	JCM 9988^T	−	−	−	−
Eubacterium multiforme	JCM 6484^T	−	−	−	−
Eubacterium nitritogenes	JCM 6485^T	−	−	−	−
Eubacterium tenue	JCM 6486^T	−	−	−	−
Eubacterium moniliforme	JCM 9990^T	−	−	−	−
Eubacterium cylindroides	JCM 10261^T	−	−	−	−
Lactobacillus plantarum	JCM 1149^T	−	−	−	−
Bifidobacterium bifidum	JCM 1255^T	−	−	−	−
Propionibacterium propionicum	JCM 5830^T	−	−	−	−
Novel strain	A2-3	+	+	+	+
Novel strain	A2-184	+	+	+	+
Novel strain	A3-99	+	+	+	+
Novel strain	A3-104	+	+	+	+
Novel strain	A3-112	+	+	+	+
Novel strain	A3-125	+	+	+	+
Novel strain	A3-252	+	+	+	+
Novel strain	A5-166	+	+	+	+
Novel strain	A5-189	+	+	+	+
Novel strain	A5-190	+	+	+	+
Novel strain	A9-162	+	+	+	+
Novel strain	RCA1-1	+	+	+	+
Novel strain	RCA1-16	+	+	+	+
Novel strain	RCA1-18	+	+	+	+
Novel strain	RCA2-10	+	+	+	+
Novel strain	RCA2-37	+	+	+	+
Novel strain	RCA58-92	+	+	+	+

139–497) (LEN-F2+LEN-R1) and 507 bp (positions 139–665) (LEN-F2+LEN-R2). The specificity of the primers was checked against published rRNA sequences. The results showed that LEN-F1, LEN-F2 and LEN-R1 did not match any published rRNA sequences; LEN-R2 has identity (<91%) to three bacterial taxa (*Afipia* genospecies, *Rhodoplanes elegans* and *Actinoplanes brasiliensis*). Since PCR was done using a combination of two primers, this was not a problem. Firstly, we used *Eubacterium lentum* JCM 9979^T DNA as a positive control and *Collinsella aerofaciens* JCM 10188^T DNA as a negative control. The PCR results showed that all primers were positive for *Eubacterium lentum* and all were negative for *Collinsella aerofaciens* (Fig. 3). Thus, these primers are all specific for *Eubacterium lentum*. Secondly, we attempted PCR with other *Eubacterium* species and other genera. The results showed that *Eubacterium lentum* was positive, but all other species were negative (Table 3). Finally, we attempted PCR with *Eubacterium lentum*-like strains isolated from human faeces (Table 1). These strains were Gram-positive, non-spore-forming, anaerobic rods that did not produce acid from arabinose, xylose, rhamnose, ribose, glucose, mannose, fructose, sucrose, maltose, cellobiose, lactose, trehalose, melibiose, raffinose, melezitose, starch, glycogen, inulin, glycerol, mannitol, sorbitol, inositol, aesculin, salicin or amygdalin. The results of PCR showed that all strains examined were amplified (Table 3). This PCR method using *Eubacterium lentum* species-specific primers was very useful for identification.

DISCUSSION

Eubacterium lentum is classified as a member of the genus *Eubacterium* because of its phenotypic characters. The genus *Eubacterium* has a broad definition and has over the years acted as a depository for a large number of phenotypically diverse species. In addition to this marked phenotypic heterogeneity, it is recognized that members of the genus *Eubacterium* are not phylogenetically homogeneous, with species dispersed among many genera, and some organisms are not related phylogenetically. The type species of the genus *Eubacterium* is *Eubacterium limosum*. It is evident from recent studies that *Eubacterium limosum*, *Eubacterium barkeri* and *Eubacterium callanderi* (Mountfort *et al.*, 1988) can form the nucleus of a redefined genus *Eubacterium*. On the basis of the characteristics of this group, a preliminary working definition of *Eubacterium sensu stricto* may be as follows.

Gram-positive, rod-shaped organisms that are non-motile and obligately anaerobic, may form endospores and are saccharolytic. The main products of glucose fermentation are butyrate, acetate, lactate and H_2. Formate or CO_2 may also be produced by some strains. The cell walls contain type B2 peptidoglycan. The G+C content of the DNA is 45–47 mol% (Willems & Collins, 1996).

From previous studies, it is well known that *Eubacterium lentum* is unique within the genus *Eubacterium* in that it has methylated menaquinone (MMK-6) in addition to menaquinone (MK-6): other species that belong to the genus *Eubacterium* do not contain menaquinone but *Eubacterium lentum* possesses significant amounts of MK-6 and MMK-6 (Fresia & Collins, 1987). The cell walls contain type A3 peptidoglycan (Schleifer & Kandler, 1972). *Eubacterium lentum* contains a branched-chain fatty acid as a major component; 66% of strains contain 12-methyl tetradecanoic acid (a-15:0) (Itoh *et al.*, 1995). Phenotypic characteristics that differentiate *Eubacterium lentum* from *Eubacterium sensu stricto* are given in Table 4.

Table 4. Characteristics that differentiate *Eubacterium lentum* and *Eubacterium sensu stricto*

Data were taken from Schleifer & Kandler (1972) unless indicated by a (Nakazawa & Hoshino, 1994) or b (Fresia & Collins, 1987). Formation is scored as: +, positive; −, negative; or (+), variable.

Characteristic	*Eubacterium lentum*	*Eubacterium sensu stricto*
Utilization of sugars	Asaccharolytic	Saccharolytic
Formation of:		
H_2	−	+
CO_2	−	(+)
Butyrate	−	+
Formate	(+)	(+)
Lactate	(+)	+
Acetate	−	+
DNA G+C content (mol %)	62[a]	45–47
Cell-wall type	A3γ	B2α
Menaquinone type	MK-6, MMK-6[b]	None[b]

In order to clarify the phylogenetic position of *Eubacterium lentum*, the 16S rRNA sequence was determined. A database search revealed that *Eubacterium lentum* belongs to the actinomycetes line. A new hierarchical classification structure for the taxa between the taxonomic levels of genus and class has been proposed for the actinomycete line of descent, as defined by analysis of a small-subunit (16S) rRNA and genes encoding this molecule (rDNA). A new class, *Actinobacteria*, including the orders *Acidimicrobiales*, *Rubrobacterales*, *Coriobacteriales*, *Sphaerobacterales*, *Actinomycetales* and *Bifidobacteriales*, has been discussed (Stackebrandt *et al.*, 1997). *Eubacterium lentum* belongs to order *Coriobacteriales*, family *Coriobacteriaceae*, and this family contains the genera *Coriobacterium* and *Atopobium* in the new classification. A phylogenetic analysis was performed including all members of the family *Coriobacteriaceae*. Our analysis shows that *Eubacterium lentum* is near to *Collinsella aerofaciens* (Kageyama *et al.*, 1999) and *Coriobacterium glomerans* (Haas & König, 1988) by this method. The two phylogenetic trees have the same topology.

The G+C content of *Eubacterium lentum* is 62 mol% (Nakazawa & Hoshino, 1994), which is different from that of the genus *Eubacterium sensu stricto* (45–47 mol%) and very similar to that of *Collinsella aerofaciens* and *Coriobacterium glomerans* (both 60–61 mol%). The G+C content is similar but the phenotypic characters were not the same.

A PCR method using species-specific primers was established as an accurate and rapid method for identification of *Eubacterium lentum*. Two sets of *Eubacterium lentum*-specific primers were designed: LEN-F1, LEN-F2, LEN-R1 and LEN-R2. These primers were checked for similarity to other species by a homology search. The result showed that no pair of primers annealed to DNA of other species. The specificity of the primers was checked using the following organisms: *Eubacterium lentum*, *Eubacterium limosum*, *Eubacterium barkeri*, *Eubacterium fossor*, *Eubacterium combesii*, *Eubacterium multiforme*, *Eubacterium nitritogenes*, *Eubacterium tenue*, *Eubacterium moniliforme*, *Eubacterium cylindroides*, *Collinsella aerofaciens*, *Coriobacterium glomerans*, *Atopobium minutum*, *Lactobacillus plantarum*, *Bifidobacterium bifidum* and *Propionibacterium propionicum* (*Coriobacteriaceae*, *Eubacterium* and other anaerobic, Gram-positive, non-spore-forming rods). The results showed that each pair of primers was positive only

for *Eubacterium lentum*; the primers are therefore useful for identification of *Eubacterium lentum*. Using this PCR system, *Eubacterium lentum* was differentiated from phylogenetically related *Coriobacteriaceae* species and phenotypically related *Eubacterium* species and anaerobic, Gram-positive, non-spore-forming rods. Finally, *Eubacterium lentum*-like strains that were isolated from human faeces were identified using this PCR method and 17 *Eubacterium lentum*-like strains were identified.

From the 16S rRNA gene sequence comparison, it was evident that the bacterium has a close phylogenetic relationship with *Collinsella aerofaciens* and *Coriobacterium glomerans*. This sequence divergence suggests that the bacteria are phylogenetically closely related, but nevertheless of different genera. In contrast to *Collinsella aerofaciens* and *Coriobacterium glomerans*, which contain type-A4 peptidoglycan, *Eubacterium lentum* contains type-A3 peptidoglycan. On the basis of the 16S rRNA sequence and the unique type-A3 peptidoglycan, we propose that *Eubacterium lentum* should be classified as the type species of a new genus, with the name *Eggerthella lenta* gen. nov., comb. nov.

Description of *Eggerthella* gen. nov.

Eggerthella (Eg.ger.thel'la. L. dim. ending *-ella*; M.L. fem. n. *Eggerthella* named after A. H. Eggerth, a microbiologist who was the first person to isolate this strain).

Cells occur singly and in pairs and short chains. Gram-positive. Obligately anaerobic. Spores and flagella are absent. The fermentation products of glucose are acetate, lactate and succinate. Hydrogen is not produced. Catalase activity is not detected. Gelatin is not liquefied, aesculin is not hydrolysed and nitrate is reduced. The cell wall contains A3γ-type peptidoglycan. Major amounts of MK-6 and MMK-6 are present. The DNA G+C content is 62 mol%. The type species is *Eggerthella lenta*. The genus *Eggerthella* is a member of the *Coriobacteriaceae* and exhibits a close phylogenetic association with the genera *Coriobacterium* and *Collinsella*.

Description of *Eggerthella lenta* [*Eubacterium lentum* (Eggerth 1935) Prévot 1938] comb. nov.

Eggerthella lenta (len'ta. L. fem. adj. *lenta* slow).

The cells are 0·2–0·4 × 0·2–2·0 μm and occur singly and in pairs and short chains. Colonies are 0·5–2·0 mm, circular, entire to erose, raised to low-convex, translucent to semi-opaque, dull to shiny, smooth and sometimes with a mottled appearance when viewed by obliquely transmitted light on EG agar plates. Gram-positive. Obligately anaerobic. Spores and flagella are absent. The fermentation products of glucose are acetate, lactate and succinate. Hydrogen is not produced. Catalase activity is not detected. Gelatin is not liquefied, aesculin is not hydrolysed and nitrate is reduced. The cells do not produce acid from arabinose, xylose, rhamnose, ribose, glucose, mannose, fructose, sucrose, maltose, cellobiose, lactose, trehalose, melibiose, raffinose, melezitose, starch, glycogen, inulin, glycerol, mannitol, sorbitol, inositol, aesculin, salicin or amygdalin. The cell wall contains A3γ-type peptidoglycan. Major amounts of MK-6 and MMK-6 are present. Fatty acid composition consists of branched-chain fatty acids as major components: 66% of strains contain 12-methyl tetradecanoic acid (a-15:0). The DNA G+C content is 62 mol%. The type strain of *Eggerthella lenta* is JCM 9979^T (= DSM 2243^T = ATCC 25559^T = NCTC 11813^T).

NOTE ADDED IN PROOF

After this paper was accepted for publication, W. G. Wade and others described the transfer of *Eubacterium lentum* to *Eggerthella lenta* gen. nov., comb. nov. [*Int J Syst Bacteriol* **49**, 595–600 (1999)]. The description of *Eggerthella lenta* (Moore *et al.*, 1971) Wade *et al.* 1999 has priority in nomenclature. However, our description of the new genus should be added to the description of Wade *et al.* (1999).

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