Lactosphaera gen. nov., a New Genus of Lactic Acid Bacteria, and Transfer of *Ruminococcus pasteurii* Schink 1984 to *Lactosphaera pasteurii* comb. nov.

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The phylogenetic position and physiology of strain KoTa2^T (T = type strain), which was previously classified as a *Ruminococcus pasteurii* strain, were studied. A determination of the 16S ribosomal DNA sequence of this taxon revealed its position within the radiation of the gram-positive lactic acid bacteria having low DNA G+C contents and that it is closely related to the genus *Carnobacterium*. L-Lactic acid was produced from glucose by a fructose-1,6-bisphosphate-activated lactate dehydrogenase, and oxygen tolerance was observed, characteristics which are consistent with assignment to this group. On the basis of its phenotypic characteristics and unique signature nucleotides, we propose that strain KoTa2 (= DSM 2381 = ATCC 35945) should be transferred to a new genus, *Lactosphaera* gen. nov., as the type strain of the species *Lactosphaera pasteurii* comb. nov.

The species *Ruminococcus pasteurii* was originally described to encompass L-tartrate-fermenting anaerobic cocci (27). This bacterium is able to ferment L-tartrate, citrate, oxaloacetate, pyruvate, and a variety of sugars, and the fermentation products have been reported to be acetate, formate, ethanol, and carbon dioxide (27). On the basis of the following characteristics of strain KoTa2^T (T = type strain) (27), the original assignment of this organism to the genus *Ruminococcus* seemed to be justified: morphology, the fermentation end products, and the G+C content of the DNA. However, our investigations showed that the type strain of *R. pasteurii*, strain KoTa2, is not physiologically or genetically related to other members of the genus *Ruminococcus*. The production of significant amounts of lactic acid, aerotolerance, and the results of a 16S ribosomal DNA (rDNA) sequence analysis indicated that this strain, previously classified as a *R. pasteurii* strain, belongs to a new taxon of lactic acid bacteria. Therefore, we describe a new genus, *Lactosphaera*, and designate the type strain of *R. pasteurii*, strain KoTa2 (= DSM 2381), the type strain of a new species, *Lactosphaera pasteurii* comb. nov.

MATERIALS AND METHODS

Strain and media. Strain KoTa2^T, which was originally described as a *R. pasteurii* strain (27), was obtained from our collection. *Ruminococcus flavefaciens* C94 (= ATCC 19208) was purchased from the American Type Culture Collection, Rockville, Md.

A freshwater mineral medium supplemented with vitamins (27) was used to cultivate strain KoTa2^T. Yeast extract was added at a concentration of 0.02% (wt/vol) unless noted otherwise. Substrate tests were performed by using sugars (D isomers) at an initial concentration of 2 mM and organic acids (L isomers) at a concentration of 10 mM. Polysaccharides were added at a concentration of 0.1% (wt/vol). All cultures were incubated at 34°C. The buffers 2-(N-morpholino)ethanesulfonic acid (MES), 3-(N-morpholino)propanesulfonic acid (MOPS), and N-(2-hydroxyethyl)-piperazine-N'-(3-propanesulfonic acid) (EPPS) were purchased from Sigma Chemical Co., St. Louis, Mo., and the pH of each medium

was adjusted to the appropriate value with NaOH. Acetate agar (which contained 15 g of agar per liter) was prepared as described by Atlas and Parks (1).

Lactic acid production and metabolic studies. High-performance liquid chromatography and gas chromatography with a thermal conductivity detector were performed as described previously (17). The isomeric form of lactic acid was determined enzymatically (2) by using D- and L-lactic acid dehydrogenases (LDHs) (Boehringer, Mannheim, Germany). Growth yields were determined as described previously (16).

Fructose-1,6-bisphosphate (Fru-1,6-P₂)-activated LDH activity was measured at 34°C as described elsewhere (21). Fru-1,6-P₂-independent LDH activity was measured by the same method except that Fru-1,6-P₂ was omitted.

Further characterization of strain KoTa2^T was carried out by using previously described methods (15–17, 25).

Peptidoglycan analysis. Cell walls were prepared and the peptidoglycan structure was determined by the methods of Schleifer and Kandler (28), modified by using thin-layer chromatography on cellulose sheets instead of paper chromatography. Briefly, 1 mg of freeze-dried cell wall material was hydrolyzed in 0.2 ml of 4 M HCl at 100°C for 16 h (total hydrolysate) or 45 min (partial hydrolysate). The diamino acids in the total hydrolysate were identified by one-dimensional chromatography by using methanol–pyridine–water–10 M HCl (32:4:7:1, vol/vol/vol/vol). The amino acids and peptides in the partial and total hydrolysates were identified by their mobilities and staining characteristics with ninhydrin spray after two-dimensional chromatography (28). The resulting “fingerprints” were compared with those of known peptidoglycan structures.

Oxygen tolerance. Oxygen was added to the desired concentration with a syringe to sealed 120-ml serum vials containing (under an N₂–CO₂ [80:20] atmosphere) 50 ml of anoxic medium supplemented with 10 mM sodium L-tartrate and 2 mM Na₂S₂O₃ but no sulfide reductant. Hemin and hematin were added from filter-sterilized (pore size, 0.2 µm) stock solutions in 2% methylamine, so that the final methylamine concentration was 0.9 mM.

Catalase (29) and superoxide dismutase (33) activities were assayed by using cell extracts that were prepared by French press treatment under anoxic conditions (18).

16S rDNA analysis. Purification of genomic DNA, in vitro amplification of 16S rRNA genes, and a direct sequence analysis of amplified DNA fragments were performed with strain KoTa2^T and *R. flavefaciens* as described elsewhere (26, 30). The derived 16S rRNA primary structure of strain KoTa2^T was added to an alignment of about 1,800 homologous sequences from bacteria. The phylogenetic affiliation of strain KoTa2^T was determined by using distance matrix and maximum-parsimony methods and a data set containing all of the available 16S rRNA sequences from gram-positive bacteria having low genomic DNA G+C contents. Subsets of the data were analyzed by using a maximum-likelihood-based treeing procedure. The reference sequences used were obtained from public databases (22, 24). The complete sequences were used to analyze close relationships, whereas the more variable sequence positions (positions at which the sequences were invariant in less than 50% of the sequences in the entire data set) were deleted to investigate remote relationships. The data analyses were performed by

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TABLE 1. Overall levels of similarity (values at lower left) of the 16S rRNA primary structures of strain KoTa2^T, *Carnobacterium* spp., selected lactic acid bacteria, and other bacteria, including members of the genus *Ruminococcus*

Organism	% Similarity ^a						
	Strain KoTa2 ^T	<i>Carnobacterium alterfunditum</i>	<i>Carnobacterium divergens</i>	<i>Carnobacterium funditum</i>	<i>Carnobacterium gallinarum</i>	<i>Carnobacterium mobile</i>	<i>Carnobacterium piscicola</i>
Strain KoTa2 ^T		95.6	95.0	95.6	95.0	95.1	95.2
<i>Carnobacterium alterfunditum</i>	94.6		96.5	97.2	96.1	96.5	96.5
<i>Carnobacterium divergens</i>	94.1	95.3		96.5	96.9	96.1	97.2
<i>Carnobacterium funditum</i>	94.2	95.9	95.5		96.1	97.7	96.5
<i>Carnobacterium gallinarum</i>	94.3	94.9	96.7	95.0		96.0	98.2
<i>Carnobacterium mobile</i>	95.1	96.5	96.3	97.7	96.1		96.3
<i>Carnobacterium piscicola</i>	94.2	95.2	96.9	95.4	98.0	96.4	
<i>Enterococcus faecalis</i>	92.0	92.6	92.6	93.8	92.1	93.6	92.0
<i>Vagococcus fluvialis</i>	92.6	92.1	92.5	91.9	91.1	93.3	91.8
<i>Lactococcus lactis</i>	87.2	87.8	87.0	87.1	86.2	87.3	86.3
<i>Streptococcus thermophilus</i>	87.0	87.5	88.2	87.6	87.3	87.4	87.5
<i>Bacillus subtilis</i>	91.2	90.3	90.2	90.0	90.3	90.1	90.3
<i>Ruminococcus flavefaciens</i>	83.0	81.1	81.7	81.7	81.1	81.5	81.3
<i>Ruminococcus gnavus</i>	76.1	75.8	75.8	75.9	74.7	76.1	75.4
<i>Ruminococcus hanseni</i>	75.2	74.8	75.5	75.1	74.5	75.7	75.0
<i>Ruminococcus productus</i>	78.7	78.1	78.6	77.5	77.3	78.5	78.0
<i>Ruminococcus torques</i>	79.1	79.1	79.2	78.9	78.2	79.7	78.8
<i>Escherichia coli</i>	78.4	78.4	78.1	77.5	77.4	78.0	77.5

^a The values on the upper right were calculated by using only data for positions that had been unambiguously determined for all members of the genus *Carnobacterium*.

using the ARB program package (23) and the treeing programs NEIGHBOR (8) and fastDNAm1 (22).

Nucleotide sequence accession numbers. The nucleotide sequences of strain KoTa2^T and *R. flavefaciens* have been deposited in the EMBL data library under accession numbers X85097 and X87150, respectively.

RESULTS

16S rDNA sequence analysis. Almost complete 16S rRNA genes of strain KoTa2^T and *R. flavefaciens* were amplified in vitro and sequenced directly. Different treeing methods and different data sets were used to reconstruct the phylogenetic relationships of strain KoTa2^T. Strain KoTa2^T is only distantly related to members of the genus *Ruminococcus* (4, 6, 7), but is closely related to members of the genus *Carnobacterium* (9, 11, 34). The overall levels of sequence similarity for strain KoTa2^T, members of the genus *Carnobacterium*, selected lactic acid bacteria, and other bacteria are shown in Table 1. The close phylogenetic relationship between strain KoTa2^T and members of the genus *Carnobacterium* is shown in Fig. 1. The position of this group among the lactic acid bacteria is shown in Fig. 2. True members of the genus *Ruminococcus* are included in Fig. 2 to show the great phylogenetic distance between these organisms and strain KoTa2^T.

FIG. 1. Phylogenetic tree derived from 16S rDNA sequence analysis, reflecting the relationships of strain KoTa2^T and members of the genus *Carnobacterium*. The tree was reconstructed by using a maximum-likelihood method. Bar = 10% estimated sequence divergence. Abbreviations: B., *Bacillus*; C., *Carnobacterium*.

Peptidoglycan structure. Purified cell walls of strain KoTa2^T contained the amino acids lysine, glutamic acid, aspartic acid, and alanine at a molar ratio of 1:1:1:2. The fingerprints of the partial hydrolysate and the presence of the hydrolysis-stable compound ε-(aminosuccinyl)-lysine are compatible only with peptidoglycan type A4α, L-Lys-D-Asp.

Oxygen tolerance. Strain KoTa2^T was able to grow in mineral medium containing 10 mM L-tartrate but no sulfide reductant (with 2 mM Na₂S₂O₃ added as a sulfur source) in the absence of yeast extract. Addition of 2.5% (vol/vol) O₂ to the headspace of static cultures inhibited growth, but this inhibition was overcome by the addition of yeast extract. Strain KoTa2^T grew well on unreduced medium supplemented with 2 mM Na₂S₂O₃ and 0.02% (wt/vol) yeast extract in the presence of O₂ partial pressures up to 10% (vol/vol). When the O₂ partial pressure was between 10 and 16% (vol/vol), growth was poor, while no growth occurred when the O₂ partial pressure was greater than 18% (vol/vol). In the presence of 5% (vol/vol) O₂, the amount of acetate produced from L-tartrate was not significantly changed, but a decrease in formate formation was detected (Table 2). There was no significant increase in the specific growth yield in the presence of oxygen.

Addition of 10 μM hemin or 10 μM hematin (dissolved in methylamine so the final methylamine concentration was 0.9 mM) or addition of 10 μM MnCl₂ did not overcome the growth inhibition caused by the presence of 2.5% (vol/vol) O₂ in the headspace in the absence of yeast extract. Methylamine at a concentration of 0.9 mM did not inhibit growth in the presence of 2.5% (vol/vol) O₂ when the medium contained 0.02% (wt/vol) yeast extract. No superoxide dismutase or catalase activity was observed in cell extracts prepared from cells grown in the presence of 2.5% (vol/vol) O₂ with 10 mM L-tartrate and 0.02% (wt/vol) yeast extract. However, growth occurred in the presence of air on Trypticase agar plates (12).

Carbon sources that support growth. Mannitol, sorbitol, and galactose supported growth of strain KoTa2^T, while ribose and glycerol did not. Strain KoTa2^T grew on starch and oat spelt xylan and grew weakly on laminarin. The following compounds did not support growth: chitin, gum karaya, carboxymethyl

FIG. 2. Consensus tree showing the phylogenetic positions of strain KoTa2^T and the genus *Carnobacterium* among the lactic acid bacteria and the distances between these taxa and the genus *Ruminococcus*. The tree was based on the results of maximum-likelihood analyses and was corrected by using the results of distance matrix and maximum-parsimony analyses. Only alignment positions which are occupied by identical nucleotides in at least 50% of all available 16S rRNA sequences from gram-positive bacteria with low DNA G+C contents were included. The triangles indicate phylogenetic groups. Bar = 10% estimated sequence divergence. Abbreviations: *E.*, *Escherichia*; *R.*, *Ruminococcus*.

cellulose, amorphous cellulose, mannan, lichenan, carrageenan, gum locust bean, pullulan, arabinogalactan, and glycogen.

Fermentation of glucose. Strain KoTa2^T produced L-lactate (in addition to formate, acetate, and ethanol) from a wide range of sugars, including glucose, fructose, maltose, lactose, sucrose, cellobiose, and sorbitol. Lactate was not formed from L-tartrate, pyruvate, or citrate.

Strain KoTa2^T was grown on 15 mM glucose, and the end products of fermentation were determined (Table 3). Significant quantities of lactate were produced (65% of the total product carbon), but very little hydrogen was produced. During growth the pH decreased from 7.2 to 5.5. The increase in lactate production was apparently not a consequence of the inclusion of yeast extract in the growth medium. The amount of lactate produced did not change when the level of yeast extract in the medium was varied from 0.01 to 0.1% (wt/vol) in the presence of 100 µg of biotin per liter. Strain KoTa2^T also produced the same amount of lactate in the absence of yeast extract.

Lactate production in the presence of a range of pH values. Strain KoTa2^T was grown on 4 mM glucose at different pH values by using heavily buffered media. The pH did not change during fermentation of glucose. The amount of lactate pro-

duced (as a proportion of the total products) increased as the acidity of the growth medium increased (Fig. 3).

The LDH activity was higher in cultures grown at more acidic pH values than in cultures grown at more basic pH values (Table 4). The LDH activity was very much lower (0 to 5%) if Fru-1,6-P₂ was omitted from the enzyme assay mixture. Cultures grown on L-tartrate or pyruvate did not produce lactate. Cells grown on pyruvate contained a Fru-1,6-P₂-activated LDH activity, and this activity was higher in cells grown under more acidic conditions than in cells grown under more basic conditions.

Additional characteristics of strain KoTa2^T. Esculin was hydrolyzed. Hydrogen in the headspace (H₂-CO₂, 80:20) did not inhibit growth on L-tartrate. Fumarate was not reduced. No growth occurred in the presence of 3% (wt/vol) NaCl, but growth did occur in the presence of 2% (wt/vol) NaCl. No growth occurred on acetate agar (pH 5.4).

DISCUSSION

16S rDNA sequence analysis. Our comparative 16S rDNA sequence analyses revealed that strain KoTa2^T is most closely related to the species of the genus *Carnobacterium* and is not

TABLE 2. Fermentation balances of strain KoTa2^T grown on L-tartrate in the absence and presence of oxygen^a

O ₂ concn in gas phase (%)	Amt of L-tartrate degraded (µmol)	Amt of products (µmol)		Amt of cell matter (mg) ^b	Amt of substrate assimilated (µmol) ^c	% Carbon recovery ^d	Ratio of formate to acetate
		Formate	Acetate				
0	500	426	441	6.66	110	106.9	0.97
5	500	166	409	6.71	111	88.3	0.41

^a The data are the means of the results of two independent experiments and were not corrected for the products and cell matter produced on yeast extract.

^b Cell dry weights were calculated from culture densities (optical densities at 650 nm) by using conversion factors which were obtained by direct gravimetric determinations performed with 1-liter cultures.

^c Calculated by using <C₄H₇O₃> as the empirical formula for cell matter.

^d Calculated by assuming that 1 mol of CO₂ was produced per mol of L-tartrate degraded; the CO₂ produced from HCOO⁻ was not included in the calculation.

TABLE 3. Fermentation balance of strain KoTa2 grown on 15 mM glucose^a

Substrate or product	Carbon balance		Hydrogen balance	
	Amt (μmol)	μmol of carbon ^b	Amt of available H (μmol) ^b	Redox value (μmol) ^c
Glucose	750	4,500	18,000	0
Lactate	867	2,601	10,404	0
Formate	343	343	686	+343
Acetate	211	422	1,688	0
Ethanol	323	646	3,876	-646
H ₂	0.1	0	0.2	-0.2
CO ₂ ^d	191	191	0	+382
Cells ^e	118	472	2,006	-59

^a The data are the means of the results of two independent experiments performed with 50-ml cultures in the presence of 0.1% (wt/vol) yeast extract. The values were corrected for the products and cell matter (9 mg · liter⁻¹) produced on 0.01% (wt/vol) yeast extract.

^b The balance was 104%.

^c The balance was 1.03 μmol.

^d The amount of CO₂ was estimated (because we used a bicarbonate-buffered medium) as follows: micromoles of CO₂ = millimoles of acetate + micromoles of ethanol - micromoles of formate.

^e Cell dry weights were calculated from culture densities (optical densities at 650 nm) by using conversion factors which were obtained by direct gravimetric determinations performed with 1-liter cultures. Values were calculated by using <C₄H₇O₃> as the empirical formula for cell matter.

related to members of the genus *Ruminococcus*. The data indicated that the *Carnobacterium* species can be considered a phylogenetic unit that does not include strain KoTa2^T. The levels of intragenomic similarity range from 94.9 to 98.0%, while the corresponding values for strain KoTa2^T and *Carnobacterium* species are 94.1 to 95.1%. There is a slight overlap of values because of the high similarity value obtained for strain KoTa2^T and *Carnobacterium mobile*. However, most of the sequences of the *Carnobacterium* species are incomplete at their 3' ends, and the sequence data for *C. mobile* do not include data for the 5'-terminal portion. Therefore, we also calculated similarity values based on only the homologous positions determined for all *Carnobacterium* spp. The values

TABLE 4. Fru-1,6-P₂-activated LDH activities in strain KoTa2^T grown on glucose and pyruvate at various pH values

Substrate	Growth pH	Buffer	% of product carbon as lactate	Fru-1,6-P ₂ -activated LDH activity (μmol · min ⁻¹ · mg of protein ⁻¹) ^a
Glucose	6.2	MES	74	0.419
	7.1	MOPS	37	0.459
	8.0	EPPS	26	0.113
Pyruvate	6.2	MES	0	0.127
	7.1	MOPS	0	0.129
	8.0	EPPS	0	0.011

^a The Fru-1,6-P₂-independent LDH activity was less than 5% of the Fru-1,6-P₂-activated LDH activity in all cases.

which we obtained are shown in Table 1 and support the congeneric status of the *Carnobacterium* spp. (similarity values, 96.0 to 98.2%) and the exclusion of strain KoTa2^T (similarity values, 95.0 to 95.6%). The separation of these taxa is also reflected by the primary structure signatures. There are a number of residues in the 16S rRNA primary structure of strain KoTa2^T that differ from the residues in the *Carnobacterium* consensus sequence (Table 5), allowing strain KoTa2^T to be differentiated from members of the genus *Carnobacterium*. The tree in Fig. 1 shows the phylogenetic unity of the genus *Carnobacterium* and the somewhat remote position of strain KoTa2^T. This tree was reconstructed by using a maximum-likelihood method, and all alignment positions were included for the calculations. The 16S rRNA sequence of *Bacillus subtilis*, a moderately closely related outgroup reference organism, was used to root the tree. The topology of the tree was evaluated by using alternative treeing methods and various data sets that differed with respect to alignment positions and reference organisms. The branching pattern was stable in most cases. The tree in Fig. 2 shows the position of the strain KoTa2^T-*Carnobacterium* cluster among the major groups of lactic acid bacteria. This tree is a consensus tree based on the results of a maximum-likelihood analysis that included 16S rRNA sequences from all members of the cluster, selected

FIG. 3. Lactate production from glucose by strain KoTa2^T at different pH values. Symbols: ●, MES buffer; □, MOPS buffer; ▲, EPPS buffer. All buffers were used at a concentration of 100 mM and were adjusted to the required pH with NaOH.

TABLE 5. Signature nucleotides in the 16S rRNA primary structure of strain KoTa2^T which are different from the nucleotides in the consensus sequence of the genus *Carnobacterium*

Position(s) ^a	Nucleotide(s) in:	
	Strain KoTa2 ^T	<i>Carnobacterium</i> consensus sequence
69	G	C
156	C	U
166	G	A
210	U	C
316 · 337	U · A	C · G
443 · 491	C · G	U · A
456	U	G
457-458 · 474-475	UG · CA	AU · AU
592 · 647	C · G	U · A
614 · 626	A · U	C · G
846	A	G
1152	A	G
1256	C	U
1278	G	U
1482	G	A

^a *E. coli* numbering system (3).

TABLE 6. Characteristics that distinguish strain KoTa2^T from the genera *Ruminococcus* and *Carnobacterium*

Taxon	Typical morphology	Peptidoglycan type	Motility	DNA G+C content (mol%)	Growth on cellulose	Major products			Oxygen tolerance	Minimum growth temp (°C)
						Lactic acid	Succinic acid	Hydrogen		
<i>Ruminococcus</i> ^a	Cocci or oval cells with pointed ends	A1γ, <i>m</i> -Dpm-direct	— ^b	39–46	+ or — ^c	—	+ or — ^c	+	—	20–30 ^e
Strain KoTa2 ^T	Cocci	A4α, L-Lys-D-Asp	—	45	—	+ ^d	—	—	+	0
<i>Carnobacterium</i> ^e	Short to medium-length rods	A1γ, <i>m</i> -Dpm-direct	+ or — ^c	33–37	—	+	—	—	+	0

^a Data from references 4, 7, and 35.^b —, negative; +, positive.^c Strain or species dependent.^d Production of lactic acid depends on the pH of the culture.^e Data from references 5, 9, 11, and 14.

representatives of the major lines of lactic acid bacteria, members of the genus *Ruminococcus*, and *Escherichia coli* as an outgroup reference organism. Given that lower levels of relationships had to be resolved, the data for variable alignment positions (positions at which the sequences were invariant in less than 50% of the sequences in the entire data set) were deleted from the data set. The tree was corrected on the basis of the results of distance matrix and maximum-parsimony analyses of about 550 16S rRNA sequences from gram-positive bacteria with low DNA G+C contents. The results of the majority of the analyses supported common roots for the genera *Vagococcus* and *Enterococcus* and the genera *Lactococcus* and *Streptococcus*. However, the branching order of these groups and the remaining major groups of lactic acid bacteria could not be determined unambiguously. This was indicated by multiple furcations in the tree. The deep branching of the genus *Ruminococcus* was supported by the results of all of the analyses which we performed.

Lactic acid production. Strain KoTa2^T formed L-lactate, as well as formate, acetate, and ethanol, from glucose in a mixed acid type of fermentation. Up to 2.0 mol of lactate was produced per mol of glucose fermented, depending on the growth pH. A shift to lactate production from acetate and ethanol production with increasing acidity has been reported in a variety of microorganisms (31), and this shift is mediated by a Fru-1,6-P₂-regulated LDH (10, 13). The Fru-1,6-P₂ activation of LDH activity explains why lactate was not produced when the organism was grown on pyruvate, since Fru-1,6-P₂ is not an intermediate of pyruvate catabolism. In pyruvate-grown cells the Fru-1,6-P₂-dependent LDH activity increased as the acidity of the medium increased, although no lactate was produced. Thus, pH appears to control induction of this enzyme, while the enzyme activity is allosterically regulated by Fru-1,6-P₂.

Oxygen tolerance. Strain KoTa2^T could grow in the presence of yeast extract under microoxic to oxic conditions, but did not contain catalase or superoxide dismutase activities. Addition of hemin, hematin, or MnCl₂ (all at a concentration of 10 μM) did not allow the organism to grow microaerobically in the absence of yeast extract; such additions are known to be components of catalase and pseudocatalase (20, 36). The basis of the microaerotolerance of strain KoTa2^T remains to be explained, and the role of yeast extract in conferring oxygen tolerance needs to be elucidated. Strain KoTa2^T apparently gained no additional metabolic energy during growth in the presence of oxygen; there were no significant changes in acetate production or specific growth yield. The amount of formate produced during growth on L-tartrate in the presence of

oxygen was greatly reduced. Transfer of electrons to oxygen by lactic acid bacteria has been described previously (11, 32), and such a transfer can produce superoxide or H₂O₂. No superoxide dismutase activity has been found in the lactic acid bacteria, and although members of the genus *Lactobacillus* can have hematin-containing catalases (36, 37) or manganese-containing pseudocatalase activities (19), members of the genus *Carnobacterium* are catalase negative (11). Lactic acid bacteria can be oxygen tolerant, and members of the genus *Carnobacterium* can even grow in the presence of air (11). In contrast, *Ruminococcus* spp. are strict anaerobes (4, 7). In this respect, strain KoTa2^T is physiologically similar to the lactic acid bacteria, particularly members of the genus *Carnobacterium*, and is not like members of the genus *Ruminococcus*.

Distinguishing features of strain KoTa2^T. Strain KoTa2^T can be distinguished from members of the genus *Ruminococcus* by the fact that it produces significant amounts of lactate under acidic conditions, by the fact that it does not produce significant amounts of hydrogen or succinate from glucose, by its oxygen tolerance, and by other characteristics (Table 6). Our comparative sequence analysis of the 16S rRNA gene showed that strain KoTa2^T is not related to members of the genus *Ruminococcus* and is closely related to members of the genus *Carnobacterium*. Strain KoTa2^T can be distinguished from members of the genus *Carnobacterium* by its coccoid morphology and DNA base composition (45 mol% G+C) (*Carnobacterium* spp. have short to medium-length, straight, slender, rod-shaped cells and DNA base compositions ranging from 33 to 37 mol% G+C) (Table 6) and by its unique signature nucleotides (Table 5). The peptidoglycan type of all previously described members of the genus *Carnobacterium* is type A1γ, *m*-Dpm-direct (5, 9, 14), while the peptidoglycan type of strain KoTa2^T is type A4α, L-Lys-D-Asp. On the basis of the data described above, we propose that strain KoTa2, previously the type strain of *R. pasteurii*, should be reclassified in a new genus, *Lactosphaera* gen. nov., as the type strain of *Lactosphaera pasteurii* comb. nov. The description below is based on data from this study and a previous study (27).

Description of *Lactosphaera* gen. nov. *Lactosphaera* (Lac.to.sphae'ra. L. gen. n. *lactis*, milk [used because of its association with lactic acid fermentation]; Gr. fem. n. *sphaira*, a sphere; N.L. fem. n. *Lactosphaera*, a lactic acid-producing sphere). *Lactosphaera* cells are gram-positive, nonmotile, nonsporulating cocci. Aminopeptidase negative. The cell wall is a single thick layer that is about 25 nm thick. The peptidoglycan type is type A4α, L-Lys-D-Asp.

Fermentative chemoorganotrophic metabolism. Some organic acids (metabolized via pyruvate) and sugars are used as

carbon and energy sources and are variously fermented to L-lactate, formate, acetate, ethanol, and CO₂. No respiratory metabolism occurs with oxygen, fumarate, nitrate, or sulfur compounds. Not generally polysaccharolytic, although some sugar polymers support growth.

As determined by a 16S rDNA analysis, the genus *Lactosphaera* belongs to the group containing gram-positive lactic acid bacteria with low DNA G+C contents and is closely related to the genus *Carnobacterium*, but is distinguished by key signature nucleotides, by its coccus-shaped cells, and by its higher DNA G+C content (45 mol%, compared with 33 to 37 mol%).

Description of *Lactosphaera pasteurii* comb. nov. *Lactosphaera pasteurii* (pas.teu'ri.i. M.L. gen. n. *pasteurii*, referring to Louis Pasteur, who probably first enriched and observed this bacterium during studies on tartrate fermentation). *L. pasteurii* cells are cocci that form pairs or small irregular packets of cells. The cells are 1.0 to 1.5 µm in diameter. No flagella are found.

Growth occurs under an air atmosphere in complex media. Growth occurs at 0 to 42°C, at pH 5.5 to 9.0, and in the presence of NaCl concentrations up to 2% (wt/vol). Catalase and superoxide dismutase activities are absent. Biotin is required as a growth factor.

Nitrate, sulfate, sulfite, thiosulfate, sulfur, and fumarate are not reduced. The presence of oxygen can result in a shift to more oxidized fermentation end products, but this is not coupled to energy conservation. No cytochromes are formed.

The following growth substrates are utilized (L isomers of organic and amino acids and D isomers of sugars unless noted otherwise): L-tartrate, pyruvate, oxaloacetate, malate, citrate, mannitol, sorbitol, glucose, galactose, mannose, L-rhamnose, fructose, maltose, lactose, sucrose, cellobiose, raffinose, trehalose, sorbose, starch, oat spelt xylan, and laminarin (weak). D-Tartrate, meso-tartrate, xylose, ribose, arabinose, malonate, succinate, DL-3-hydroxybutyrate, lactate, amino acids, alcohols, chitin, gum karaya, carboxymethyl cellulose, amorphous cellulose, mannan, lichenan, gum locust bean, pullulan, arabinogalactan, and glycogen are not utilized.

L-Tartrate, pyruvate, and citrate are fermented to acetate and formate (and CO₂). Glucose and other carbohydrates are fermented to L-lactate, acetate, formate, and ethanol. Lactate production increases under acidic growth conditions and is mediated by a Fru-1,6-P₂-activated LDH. LDH induction is controlled by pH. Succinate production and H₂ production are insignificant.

Negative for oxidase activity, urease activity, indole production, sulfide production from cysteine, and gelatin hydrolysis. Esculin is hydrolyzed. No growth occurs on acetate agar (pH 5.4).

The DNA base composition of strain KoTa2^T is 45 mol% G+C.

The type strain is KoTa2 (= DSM 2381 = ATCC 35945), which was isolated from anoxic digester sludge.

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