

# INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY

Volume 44

July 1994

No. 3

## ORIGINAL PAPERS RELATING TO SYSTEMATIC BACTERIOLOGY

- The Phloem-Limited Bacterium of Greening Disease of Citrus Is a Member of the  $\alpha$  Subdivision of the *Proteobacteria*.** Sandrine Jagoueix, Joseph-Marie Bové, and Monique Garnier..... 379-386
- Phylogenetic Relationships between Some Members of the Genera *Neisseria*, *Acinetobacter*, *Moraxella*, and *Kingella* Based on Partial 16S Ribosomal DNA Sequence Analysis.** M. C. Enright, P. E. Carter, I. A. MacLean, and H. McKenzie..... 387-391
- Photosynthetic Symbionts of *Aeschynomene* spp. Form a Cluster with *Bradyrhizobia* on the Basis of Fatty Acid and rRNA Analyses.** Rolando B. So, Jagdish K. Ladha, and J. Peter W. Young..... 392-403
- Comparison of the *Sma*I-Digested Chromosomes of *Staphylococcus epidermidis* and the Closely Related Species *Staphylococcus capitis* and *Staphylococcus caprae*.** Carol G. George and Wesley E. Kloos..... 404-409
- Pseudomonas flavescens* sp. nov., Isolated from Walnut Blight Cankers.** D. C. Hildebrand, N. J. Palleroni, M. Hendson, J. Toth, and J. L. Johnson..... 410-415
- Phylogenetic Analysis and Assessment of the Genera *Vibrio*, *Photobacterium*, *Aeromonas*, and *Plesiomonas* Deduced from Small-Subunit rRNA Sequences.** Raymond Ruimy, Violette Breittmayer, Philippe Elbaze, Bénédicte Lafay, Odile Boussemart, Michel Gauthier, and Richard Christen..... 416-426
- Phylogenetic Positions of Novel Aerobic, Bacteriochlorophyll *a*-Containing Bacteria and Description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov.** Vladimir Yurkov, Erko Stackebrandt, Andrew Holmes, John A. Fuerst, P. Hugenholtz, Jochen Golecki, Nasser Gad'on, Vladimir M. Gorlenko, Elena I. Kompantseva, and Gerhart Drews..... 427-434
- Lactobacillus kefirgranum* sp. nov. and *Lactobacillus parakefir* sp. nov., Two New Species from Kefir Grains.** S. Takizawa, S. Kojima, S. Tamura, S. Fujinaga, Y. Benno, and T. Nakase..... 435-439
- Phylogenetic Classification of Phytopathogenic Mollicutes by Sequence Analysis of 16S Ribosomal DNA.** Erich Seemüller, Bernd Schneider, Rainer Mäurer, Ulrich Ahrens, Xavier Daire, Heike Kison, Karl-Heinz Lorenz, Giuseppe Firrao, Lidon Avinent, Barbara B. Sears, and Erko Stackebrandt..... 440-446
- Flavobacterium scophthalmum* sp. nov., a Pathogen of Turbot (*Scophthalmus maximus* L.).** M. Mudarris, B. Austin, P. Segers, M. Vancanneyt, B. Hoste, and J. F. Bernardet..... 447-453
- Identification of the *Staphylococcus sciuri* Species Group with *Eco*RI Fragments Containing rRNA Sequences and Description of *Staphylococcus vitulus* sp. nov.** John A. Webster, Tammy L. Bannerman, Romeo J. Hubner, Deborah N. Ballard, Eileen M. Cole, James L. Bruce, Franz Fiedler, Karin Schubert, and Wesley E. Kloos..... 454-460
- Phenotypic and Genotypic Characterization of *Bradyrhizobia* Nodulating the Leguminous Tree *Acacia albida*.** Nicolas Dupuy, Anne Willems, Bruno Pot, Dirk Dewettinck, Ilse Vandenbrouaene, Gloria Maestrojuan, Bernard Dreyfus, Karel Kersters, Matthew David Collins, and Monique Gillis..... 461-473

Continued on following page

<b><i>Nocardiosis halophila</i> sp. nov., a New Halophilic Actinomycete Isolated from Soil.</b> Amira Mahmoud Al-Tai and Ji-Sheng Ruan .....	474-478
<b><i>Mycoplasma auris</i> sp. nov., <i>Mycoplasma cottewii</i> sp. nov., and <i>Mycoplasma yeatsii</i> sp. nov., New Sterol-Requiring Mollicutes from the External Ear Canals of Goats.</b> A. J. DaMassa, J. G. Tully, D. L. Rose, D. Pitcher, R. H. Leach, and G. S. Cottew .....	479-484
<b>Transfer of Polychlorophenol-Degrading <i>Rhodococcus chlorophenolicus</i> (Apajalahti et al. 1986) to the Genus <i>Mycobacterium</i> as <i>Mycobacterium chlorophenolicum</i> comb. nov.</b> Max M. Häggblom, Liisa J. Nohynek, Norberto J. Palleroni, Kaarina Kronqvist, Eeva-Liisa Nurmiäho-Lassila, Mirja S. Salkinoja-Salonen, Stefan Klatte, and Reiner M. Kroppenstedt .....	485-493
<b>Phylogenetic Evidence for Transfer of Pentachlorophenol-Mineralizing <i>Rhodococcus chlorophenolicus</i> PCP-I<sup>T</sup> to the Genus <i>Mycobacterium</i>.</b> Maria Briglia, Rik I. L. Eggen, Dirk J. Van Elsas, and Willem M. De Vos .....	494-498
<b>Classification of <i>Pseudomonas diminuta</i> Leifson and Hugh 1954 and <i>Pseudomonas vesicularis</i> Büsing, Döll, and Freytag 1953 in <i>Brevundimonas</i> gen. nov. as <i>Brevundimonas diminuta</i> comb. nov. and <i>Brevundimonas vesicularis</i> comb. nov., Respectively.</b> P. Segers, M. Vancanneyt, B. Pot, U. Torck, B. Hoste, D. Dewettinck, E. Falsen, K. Kersters, and P. De Vos .....	499-510
<b><i>Rhizobium ciceri</i> sp. nov., Consisting of Strains That Nodulate Chickpeas (<i>Cicer arietinum</i> L.).</b> Sarah M. Nour, Maria P. Fernandez, Philippe Normand, and Jean-Claude Cleyet-Marel .....	511-522
<b>Phylogenetic Analysis of a New LL-Diaminopimelic Acid-Containing Coryneform Bacterium from Herbage, <i>Nocardioides plantarum</i> sp. nov.</b> M. D. Collins, S. Cockcroft, and Sally Wallbanks .....	523-526
<b>Evolutionary Relationships among Eubacterial Groups as Inferred from GroEL (Chaperonin) Sequence Comparisons.</b> Alejandro M. Viale, Adrián K. Arakaki, Fernando C. Soncini, and Raúl G. Ferreyra .....	527-533
<b>Isolation and Characterization of <i>Halothermothrix orenii</i> gen. nov., sp. nov., a Halophilic, Thermophilic, Fermentative, Strictly Anaerobic Bacterium.</b> J.-L. Cayol, B. Ollivier, B. K. C. Patel, G. Prensier, J. Guezennec, and J.-L. Garcia .....	534-540
<b>Classification of Leptospire of the Pyrogenes Serogroup Isolated from Cattle in Zimbabwe by Cross-Agglutinin Absorption and Restriction Fragment Length Polymorphism Analysis.</b> Sara B. Feresu, Carole A. Bolin, Hans Korver, and Wiepko J. Terpstra .....	541-546
<b>Pulsed-Field Gel Electrophoresis of Genomic Digests of <i>Thermus</i> Strains and Its Implications for Taxonomic and Evolutionary Studies.</b> A. G. Rodrigo, K. M. Borges, and P. L. Bergquist .....	547-552
<b>Phylogeny of <i>Helicobacter</i> Isolates from Bird and Swine Feces and Description of <i>Helicobacter pametensis</i> sp. nov.</b> Floyd E. Dewhirst, Charles Seymour, Gayle J. Fraser, Bruce J. Paster, and James G. Fox .....	553-560
<b>Fatty Acid and Protein Profiles of <i>Streptomyces scabies</i> Strains Isolated in Eastern Canada.</b> Eric Paradis, Claudia Goyer, Nancy C. Hodge, Richard Hogue, Robert E. Stall, and Carole Beaulieu .....	561-564
<b><i>Haloanaerobium salsugo</i> sp. nov., a Moderately Halophilic, Anaerobic Bacterium from a Subterranean Brine.</b> V. K. Bhupathiraju, A. Oren, P. K. Sharma, R. S. Tanner, C. R. Woese, and M. J. McInerney .....	565-572
<b><i>Methanobus taylorii</i> sp. nov., a New Methylophilic, Estuarine Methanogen.</b> Ronald S. Oremland and David R. Boone .....	573-575

<b>Emendation of the Description of <i>Acidaminococcus fermentans</i>, a <i>trans</i>-Aconitate- and Citrate-Oxidizing Bacterium.</b> Gregory M. Cook, Frederick A. Rainey, Guangjiong Chen, Erko Stackebrandt, and James B. Russell.....	576–578
<b>Transfer of <i>Propionibacterium innocuum</i> Pitcher and Collins 1991 to <i>Propioniferax</i> gen. nov. as <i>Propioniferax innocua</i> comb. nov.</b> Akira Yokota, Tomohiko Tamura, Mariko Takeuchi, Nobert Weiss, and Erko Stackebrandt.....	579–582
<b>Phylogenetic Analysis of Species of the <i>meso</i>-Diaminopimelic Acid-Containing Genera <i>Brevibacterium</i> and <i>Dermabacter</i>.</b> Junpeng Cai and Matthew D. Collins.....	583–585

MATTERS RELATING TO THE INTERNATIONAL COMMITTEE ON  
SYSTEMATIC BACTERIOLOGY

*Taxonomic Notes*

<b>Synonymy of <i>Enterobacter cancerogenus</i> (Urošević 1966) Dickey and Zumoff 1988 and <i>Enterobacter taylorae</i> Farmer et al. 1985 and Resolution of an Ambiguity in the Biochemical Profile.</b> H. C. Schønheyder, K. T. Jensen, and W. Frederiksen .....	586–587
<b>An Aid to Formation of Bacterial Names: Chemical Terminology and Microbiological Nomenclature.</b> R. E. Buchanan .....	588–590

*Request for an Opinion*

<b>Phylogenetic Placement of <i>Sarcina ventriculi</i> and <i>Sarcina maxima</i> within Group I <i>Clostridium</i>, a Possible Problem for Future Revision of the Genus <i>Clostridium</i>.</b> A. Willems and M. D. Collins .....	591–593
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*Minutes*

<b>ICSB Subcommittee on the Taxonomy of Staphylococci and Streptococci.</b> .....	594
---	-----

*Validation List*

<b>Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB. List No. 50.</b> .....	595
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*Notification List*

<b>Notification that New Names and New Combinations Have Appeared in Volume 44, No. 2, of the IJSB.</b> .....	596
---	-----

ERRATUM

<b>A New Serovar in the Grippotyphosa Serogroup Comprising Leptospiral Isolates from Different Regions.</b> J. L. Herrmann, P. Bakoss, H. Korver, A. A. Bulu, E. Bellenger, W. J. Terpstra, I. Saint Girons, and G. Baranton.....	597
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*AUTHOR'S CORRECTION*

**16S Ribosomal DNA Sequences of Anaerobic Cocci and Proposal of *Ruminococcus  
hansenii* comb. nov. and *Ruminococcus productus* comb. nov.** Takayuki  
Ezaki, Na Li, Yasuhiro Hashimoto, Hiroaki Miura, and Hiroyuki  
Yamamoto .....

# Identification of the *Staphylococcus sciuri* Species Group with *EcoRI* Fragments Containing rRNA Sequences and Description of *Staphylococcus vitulus* sp. nov.

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Strains of a new species, *Staphylococcus vitulus*, were isolated from food and a variety of mammals. This species was recognized on the basis of the results of an analysis of genomic *EcoRI* fragments containing portions of the rRNA operons. The patterns of hybridized fragments obtained from strains belonging to the new taxon were sorted into a distinguishable cluster and were distinct from the *Staphylococcus lentus* and *Staphylococcus sciuri* patterns. The results of DNA-DNA hybridization reactions demonstrated that strains in this cluster were more closely related to *S. lentus* and *S. sciuri* than to other *Staphylococcus* species and yet were significantly different. While these strains had some of the phenotypic characteristics of the *S. sciuri* species group, the newly recognized taxon could be distinguished by its very small colonies on P agar, absence of alkaline phosphatase activity, and lack of acid production from L-arabinose, maltose, N-acetylglucosamine, D-mannose, and raffinose. The type strain of the new species is strain DD 756 (= ATCC 51145).

A general method for distinguishing bacterial species by using restriction fragments containing portions of their rRNA operons has been described previously (12, 26, 31). This method has been applied to the genus *Staphylococcus* (8, 9, 29) and recently was recommended as a way to distinguish a newly described staphylococcus from previously described taxa (7).

In this study, the electrophoretic patterns of restriction fragments labeled by hybridization with an rRNA operon from *Escherichia coli* were used to characterize organisms belonging to the *Staphylococcus sciuri* species group. When the patterns were sorted on the basis of similarity by using correlation values, clusters of strains identified as *S. sciuri* and *Staphylococcus lentus* were formed. We also distinguished another cluster of novobiocin-resistant, oxidase-positive staphylococci. This third taxon and its relationship to the *S. sciuri* species group are described in this paper.

## MATERIALS AND METHODS

**Bacterial strains.** In this study, strains were identified by their DuPont numbers. Table 1 shows the strains which we studied, other designations of some strains, the species or subspecies to which each strain belongs, and the source of each strain.

**Characteristic determinations.** The following characteristics were determined as previously described (18, 19, 21, 22): colony morphology and pigment, motility, anaerobic growth in thioglycolate broth, catalase activity, acetylmethylcarbinol (acetoin) production, nitrate reduction, tube coagulase activity, clumping factor, hemolysis of bovine blood, carbohydrate reactions, and susceptibility to various antibiotics. Clumping factor and protein A were detected with a Staph Latex kit (Remel, Lenexa, Kans.). The oxidase test was performed by using a Microdase disk (Remel) (10). Pyrrolidonyl arylamidase

activity was determined by using the Pyr broth and Pyr reagent of Carr-Scarborough Microbiologicals (Stone Mountain, Ga.) for identification of group A streptococci and enterococci (13). Esculin hydrolysis was determined on Aesculin agar (Carr-Scarborough Microbiologicals). Heat-stable nuclease activity was analyzed by using thermonuclease agar supplemented with toluidine blue (Remel) according to the manufacturer's instructions. Ornithine decarboxylase activity was determined by using a modification of the test of Moeller (25), as described in the *Manual of Clinical Microbiology* (18). Alkaline phosphatase, urease,  $\beta$ -galactosidase,  $\beta$ -glucosidase, and  $\beta$ -glucuronidase activities and arginine utilization were analyzed by using the API STAPH-IDENT system (bio-Merieux Vitek, Hazelwood, Mo.). Additional biochemical profile data were obtained by using the STAPH Trac system (bio-Merieux Vitek).

**DNA-DNA hybridization.** DNA was isolated and purified by using the procedures of Brenner and coworkers (5), as modified by Kloos and Wolfshohl (23). DNA-DNA hybridization reactions were performed under stringent (70°C) and optimal (55°C) conditions, and single- and double-stranded DNAs were separated by using the batch procedure for determining the extent of hybridization and thermal elution of DNA from hydroxyapatite (5).

**Patterns: cell and DNA processing.** Cells from 3 ml of broth were isolated and lysed in 10 mM Tris-HCl-10 mM sodium chloride-50 mM EDTA (pH 8.0) by sequentially adding 30  $\mu$ g of N-acetylmuramidase, 600  $\mu$ g of lysozyme, 25 U of lyso-staphin, and 10 U of RNase (total volume, 70  $\mu$ l). Following incubation, 800  $\mu$ g of achromopeptidase in 40  $\mu$ l of water was added. After a second incubation, 126  $\mu$ l of a 10% sodium dodecyl sulfate (SDS) solution and 1.26 mg of proteinase K (concentration, 10 mg/ml) were added. The genomic DNA was isolated by phenol-chloroform-water extraction and ethanol precipitation. The DNA was digested to completion with *EcoRI*.

**Patterns: electrophoresis, transfer, denaturation, and UV cross-linking.** The DNA fragments were separated by electrophoresis in a 0.8% agarose gel in a minigel apparatus. The

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TABLE 1. *Staphylococcus* strains used, species designations, and sources of strains

Strain <sup>a</sup>			Bacterial species or subspecies	Source
DuPont no.	Kloos no.	Other designation		
DD 756 <sup>T</sup>		ATCC 51145 <sup>T b</sup>	<i>S. vitulus</i>	Ground lamb
DD 763		ATCC 51162 <sup>b</sup>	<i>S. vitulus</i>	Processed chicken
DD 768			<i>S. vitulus</i>	Beef, ground chuck
DD 771		ATCC 51163 <sup>b</sup>	<i>S. vitulus</i>	Beef, ground chuck
DD 791			<i>S. sciuri</i>	Veal leg, sliced
DD 852 <sup>T</sup>	GH137 <sup>T</sup>	ATCC 29974 <sup>T</sup>	<i>S. cohnii</i> subsp. <i>cohnii</i>	Human
DD 853 <sup>T</sup>		ATCC 43958 <sup>T</sup>	<i>S. equorum</i>	Horse
DD 854 <sup>T</sup>		ATCC 35539 <sup>T</sup>	<i>S. gallinarum</i>	Poultry
DD 857 <sup>T</sup>	SC210 <sup>T</sup>	ATCC 43959 <sup>T</sup>	<i>S. kloosii</i>	Eastern gray squirrel
DD 863 <sup>T</sup>		ATCC 12600 <sup>T</sup>	<i>S. aureus</i>	Human
DD 864 <sup>T</sup>		ATCC 14990 <sup>T</sup>	<i>S. epidermidis</i>	Human
DD 866 <sup>T</sup>		ATCC 15305 <sup>T</sup>	<i>S. saprophyticus</i>	Human
DD 869 <sup>T</sup>	KL162 <sup>T</sup>	ATCC 29971 <sup>T</sup>	<i>S. xylosus</i>	Human
DD 871 <sup>T</sup>		ATCC 43957 <sup>T</sup>	<i>S. arlettae</i>	Poultry
DD 1078		CFDRA MDW58	<i>S. vitulus</i>	Minced beef
DD 4201	OA542		<i>S. lentus</i>	Domestic sheep
DD 4202	K-15		<i>S. lentus</i>	Dairy goat
DD 4203 <sup>T</sup>	K-21 <sup>T</sup>	ATCC 29070 <sup>T</sup>	<i>S. lentus</i>	Dairy goat
DD 4204	K-6		<i>S. lentus</i>	Dairy goat
DD 4277 <sup>T</sup>	SC 116 <sup>T</sup>	ATCC 29062 <sup>T</sup>	<i>S. sciuri</i>	Eastern gray squirrel
DD 4522	H4F28		<i>S. vitulus</i>	Yearling horse
DD 4523	V51	ATCC 51161 <sup>b</sup>	<i>S. vitulus</i>	Pine vole
DD 4524	VE 2		<i>S. vitulus</i>	Raw veal trimmings
DD 4525	VE 15		<i>S. vitulus</i>	Raw veal trimmings
DD 4535	RM112		<i>S. sciuri</i>	Black rat
DD 4751	BH2		<i>S. sciuri</i>	Beef head
DD 4756	BT12		<i>S. sciuri</i>	Beef trimmings
DD 4753	VE 23		<i>S. vitulus</i>	Raw veal trimmings
DD 5310	BC5F4		<i>S. lentus</i>	Beef cattle
DD 6049	TT4m1		<i>S. lentus</i>	Bottlenose dolphin
DD 6065	GMWg12		<i>S. vitulus</i>	Pilot whale

<sup>a</sup> ATCC, American Type Culture Collection, Rockville, Md.; CFDRA, Campden Food and Drink Research Association, Campden, United Kingdom.

<sup>b</sup> This strain was submitted to the American Type Culture Collection during preparation of the manuscript.

separated DNA fragments were transferred from the agarose gel to a nylon membrane (type NT4HY; Micron Separations, Westborough, Mass.). The DNA was then denatured, and the membrane was dried. The DNA was cross-linked to the nylon membrane by using UV light.

**Patterns: probe preparation, hybridization, and detection.** A plasmid containing the rRNA operon (*rrnB*) from *E. coli* (6) was linearized with *EcoRI* and labeled by using a sulfonation reaction (30).

The hybridization cocktail contained 125 µg of sonicated and denatured salmon sperm DNA per ml, 0.5 M sodium chloride, and 1% SDS. For each membrane, 1.5 µg of the sulfonated DNA was denatured and combined with 6 ml of hybridization cocktail. After overnight hybridization at 66°C, the membrane was washed with a 0.5 M sodium chloride-1% SDS solution at 66°C before it was dried.

The modified DNA probe was detected by using a conjugate consisting of anti-sulfonated DNA monoclonal antibody and alkaline phosphatase (15, 16). The monoclonal antibody (Orgenics, Yavne, Israel) was activated with *N*-succinimidyl-4-(*N*-maleimidemethyl)cyclohexane-1-carboxylate. Sulfhydryl groups were created on the alkaline phosphatase by using *N*-succinimidyl-5-acetylthiacetate and were deprotected with hydroxylamine. Conjugation of monoclonal antibody-maleimide with alkaline phosphatase containing sulfhydryl groups was accomplished by incubating the preparation in the dark. The conjugate was purified on a Zorbax GF-250 column.

After treatment with blocking buffer (30 g of skim milk powder [Difco Laboratories, Detroit, Mich.] per 100 ml of 25

mM NaCl-50 mM Tris-HCl [pH 7.5]-1 mM EDTA-0.3% Tween 20) and with the conjugate solution, the membrane was washed with assay buffer (50 mM sodium bicarbonate-carbonate, 1 mM magnesium chloride; pH 9.5). After the final wash fluid was decanted, 20 ml of assay buffer and 220 µl of chemiluminescent substrate solution PPD (10 mg/ml; Lumigen, Detroit, Mich.) were added to each membrane preparation. The membrane was removed from the solution, dried, and attached to a plastic frame, and chemiluminescent images were recorded electronically by using a high-sensitivity, super-cooled Star One camera (Photometrics, Tucson, Ariz.) in a dark environment. The images were stored on a Macintosh IICx computer (Apple Computer, Cupertino, Calif.).

**Patterns: data processing.** For each membrane image, the software located the lane positions, reduced the background and noise, scaled each lane's image intensity, and used the data from the lanes containing DNA standards of known sizes to normalize the band positions. The normalized position and intensity profile for each lane, referred to as a pattern, was then stored as an individual record consisting of 512 bytes in a data base.

Additional custom software based on the method of Hubner (14) was used to analyze the levels of correlation between pairs of patterns. This software used the 512 intensity values for each lane as coordinates in a Euclidean, 512-dimensional space. Each pattern represented a single point in the 512-dimensional space. Each pair of patterns was compared by measuring the angle between the pair of lines constructed from the origin of the 512-dimensional space to each of the two points created

TABLE 2. Results of hybridization of staphylococcal DNAs with [*methyl-<sup>3</sup>H*]thymidine-labeled DNAs

Species or subspecies	Pattern type <sup>a</sup>	Strain	% Relative binding with labeled DNA from:							
			DD 756 <sup>T</sup>		DD 771		DD 4525		DD 4523	
			55°C	70°C	55°C	70°C	55°C	70°C	55°C	70°C
<i>S. vitulus</i>	dd 756	DD 756 <sup>T</sup>	100	100	81	84	74	81	81	79
	dd 756	DD 4753	87	93	97	93	78	84	76	80
	dd 756	DD 6065	74	82	81	88	NT <sup>b</sup>	NT	NT	NT
	dd 771	DD 771	74	77	100	100	86	81	86	79
	dd 771	DD 1078	76	76	84	84	88	84	80	81
	dd 4522	DD 4522	75	75	88	81	88	83	85	84
	dd 4522	DD 4525	74	83	87	83	100	100	84	88
	dd 4523	DD 4523	79	76	85	81	81	82	100	100
<i>S. sciuri</i>		DD 4277 <sup>T</sup>	46	10	53	14	NT	NT	NT	NT
<i>S. lentus</i>		DD 4203 <sup>T</sup>	38	7	42	15	NT	NT	NT	NT
<i>S. saprophyticus</i>		DD 866 <sup>T</sup>	14	7	25	9	NT	NT	NT	NT
<i>S. cohnii</i> subsp. <i>cohnii</i>		DD 852 <sup>T</sup>	19	7	28	9	NT	NT	NT	NT
<i>S. xylosus</i>		DD 869 <sup>T</sup>	15	5	27	9	NT	NT	NT	NT
<i>S. kloosii</i>		DD 857 <sup>T</sup>	14	5	29	8	NT	NT	NT	NT
<i>S. equorum</i>		DD 853 <sup>T</sup>	16	6	25	9	NT	NT	NT	NT
<i>S. arlettae</i>		DD 871 <sup>T</sup>	17	8	28	10	NT	NT	NT	NT
<i>S. gallinarum</i>		DD 854 <sup>T</sup>	13	8	14	5	NT	NT	NT	NT

<sup>a</sup> Each pattern type was named after the strain with the lowest DuPont Company number that exhibited that pattern.

<sup>b</sup> NT, not tested.

from the two patterns being compared. Similar patterns had angles approaching zero degrees and cosines approaching unity (one). While the correlation was defined as the cosine of the angle, in this work we routinely used the correlation value squared.

**Cell wall analysis.** Staphylococci were grown to the stationary phase in a medium containing (per liter of distilled water) 10 g of tryptone, 5 g of yeast extract, 5 g of glucose, and 5 g of NaCl (pH 7.3). The cells were harvested by centrifugation at 20,000 × *g* for 20 min at 4°C and were disintegrated by shaking the preparation with glass beads. Cell walls were purified with 4% SDS by using the procedures of Glauner et al. (11) to determine peptidoglycan composition and teichoic acids. For total amino acid analysis cell walls purified with SDS were hydrolyzed with 4 N HCl for 16 h at 100°C. The levels of amino acids and amino sugars were determined by using a model LC6001 amino acid analyzer (Biotronik, Maintal, Germany). The peptidoglycan type was determined on the basis of the molar ratio of glutamic acid to L-lysine to L-serine to L-alanine to glycine (28). Teichoic acids were extracted from cell walls purified with SDS by the method of Kaya et al. (17). The teichoic acids were purified by ion-exchange chromatography on a column (1.6 by 17 cm) filled with DEAE-Sephacel (Pharmacia, Uppsala, Sweden), using a flow rate of 20 ml/h, a base mobile phase consisting of 0.01 M Tris-HCl (pH 7.0), and a linear 0 to 1 M NaCl gradient. The teichoic acids were hydrolyzed with 60% (wt/vol) hydrofluoric acid for 16 h at 0°C (3). Nonphosphorylated teichoic acid fragments were separated on a column (1.5 by 90 cm) filled with Bio-Gel P-2 (Pharmacia) by using a distilled water mobile phase and a flow rate of 8 ml/h. The sugars in the fractions were determined by gas-liquid chromatography, and glycerol was detected enzymatically by the method of Bergmeyer (4). The sugars were completely hydrolyzed with 2 N HCl for 3 h at 100°C (3). The hydrolysates were derivatized as described by Albersheim et al. (1) and were subjected to gas-liquid chromatography by using a Packard model GC 438 gas chromatograph equipped with a flame ionization detector (Packard Instrument Co., Meriden, Conn.) and a column (2 by 1,000 mm) filled with 3% SP 2340 on 100/200 Supelcoport (Supelco, Bellefonte, Pa.). The gas-

liquid chromatography column was equilibrated at 140°C and was kept at that temperature for the first 2 min of each analysis. The temperature was increased at a rate of 6°C/min until it reached 270°C, where it was kept for an additional 3 min. Phosphate levels were determined by the method of Ames (2).

**DNA base composition.** The guanine-plus-cytosine content of DNA was determined by A. G. Steigerwalt in the laboratory of D. J. Brenner, Centers for Disease Control, Atlanta, Ga., by using the thermal denaturation method of Marmur and Doty (24).

## RESULTS AND DISCUSSION

**DNA-DNA hybridization.** The DNA relationships among *Staphylococcus vitulus* strains that represented different pattern types and between *S. vitulus* and other *Staphylococcus* species are shown in Table 2.

In DNA-DNA hybridization reactions performed under optimal (55°C) and stringent (70°C) conditions, *S. vitulus* strains exhibited relatively high levels of DNA similarity (82% ± 6% at 55°C and 82% ± 4% at 70°C [means ± standard deviations]). No significant differences in levels of DNA similarity were found between *S. vitulus* strains that represented different pattern types. However, the levels of DNA relatedness between the new species and other members of the *S. sciuri* species group, including *S. lentus* and *S. sciuri*, were significantly lower. For *S. lentus*, the levels of DNA similarity were 40% ± 2% at 55°C and 11% ± 4% at 70°C. For *S. sciuri*, the levels of DNA similarity were 50% ± 4% at 55°C and 12% ± 2% at 70°C. The levels of DNA relatedness between *S. vitulus* and other novobiocin-resistant *Staphylococcus* species that did not belong to the *S. sciuri* species group were even lower.

**Patterns.** The raw image from a typical agarose gel generated by using enzyme-triggered chemiluminescence and captured with a customized charge-coupled device camera is shown in Fig. 1. The lanes that are not labeled with a species name were used to separate DNA standards whose sizes were known. The positions of the standard bands were used to

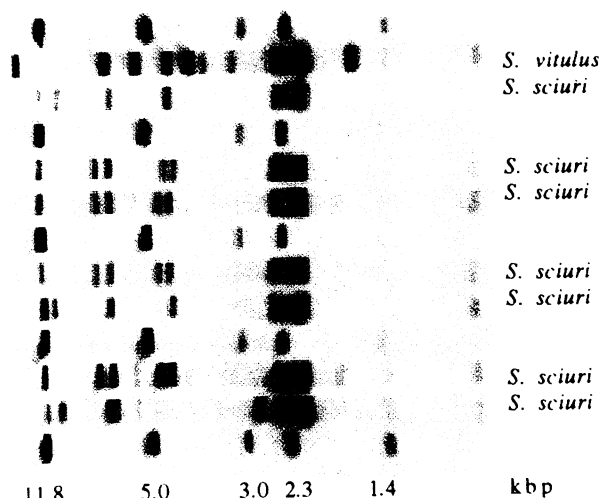


FIG. 1. Electronically recorded image of a membrane containing *EcoRI* fragments hybridized with a plasmid containing the *rmlB* rRNA operon of *E. coli*.

correct for both lane-to-lane and gel-to-gel variations in band position.

A processed image of some of the patterns determined during this study is shown in Fig. 2. The displayed width of each lane pattern was arbitrarily chosen for Fig. 2. The patterns were arranged to maximize the squared correlation values for adjacent patterns in the display. This sorting revealed that the *S. vitulus* patterns were most similar to the *S. lentus* and *S. sciuri* patterns and were different from the patterns obtained with the more than 200 other species in our data base. *S. vitulus*, *S. lentus*, and *S. sciuri* could be differentiated by their patterns either by visually comparing the patterns or by computer analysis.

Visually, the qualitative differences among the patterns of the three species were obvious. The *S. vitulus* patterns revealed sets of conserved, frequently occurring restriction fragments that were found in different strains of *S. vitulus* but were not conserved as a group in strains of other species.

Patterns that were identical (within the reproducibility of the method) were considered members of the same pattern type. Multiple strains belonging to the same species could have the same pattern type. Each pattern type was named after the lowest-numbered DuPont strain that produced that type of pattern. The 11 *S. vitulus* strains examined produced four distinguishable types of patterns, dd 756, dd 771, dd 4522, and dd 4523.

Computer analysis provided a way to measure the levels of similarity of the patterns by using the squared correlation values obtained from the 512-dimensional space. All of the *S. vitulus* patterns of the same pattern type (for example, dd 756) had squared correlation values with each other that were greater than 0.85. When each of the four *S. vitulus* pattern types was correlated with its nearest *S. vitulus* neighbors in Fig. 2, the squared correlation values ranged from 0.71 to 0.79. No *S. vitulus* pattern had a squared correlation value with any *S. lentus* pattern that was greater than 0.56 or a squared correlation value with any *S. sciuri* pattern that was greater than 0.46.

Figures 3 and 4 show two-dimensional representations of the 512-dimensional space. The patterns of more than 5,500 strains, including members of approximately 200 species, were

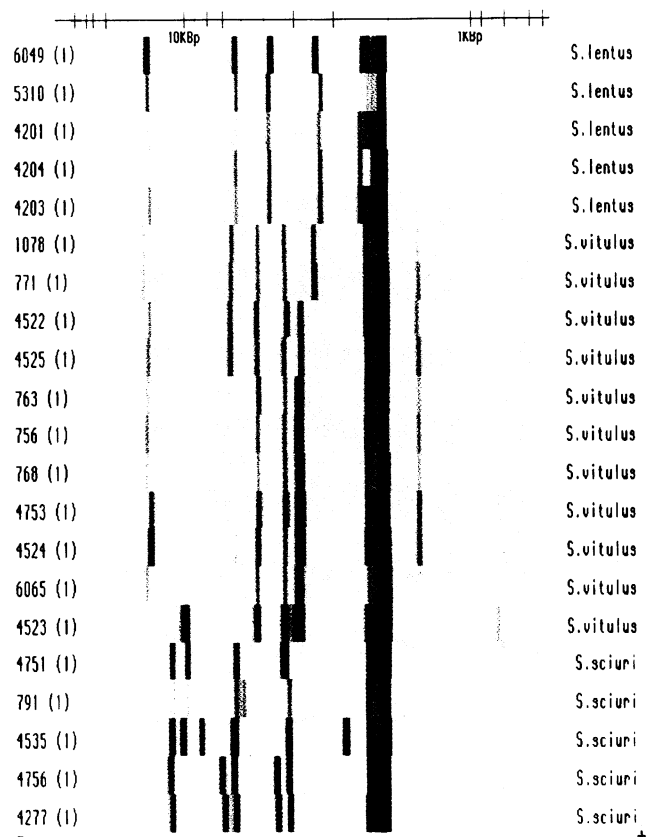


FIG. 2. Patterns of *EcoRI* fragments containing rRNA sequences derived from image data. The image data for each lane were processed to normalize band positions relative to standards, to reduce background, and to scale the band intensity. The pattern numbers are shown on the left, and the species names are shown on the right.

each defined by a single labeled point in Fig. 3 and 4. In each figure, the squared correlation values with the two reference patterns defined the two dimensions. For Fig. 3 we used the nomenclatural type strain of *S. lentus*, DD 4203, and the nomenclatural type strain of *S. vitulus*, DD 756, as the reference strains. For Fig. 4 we used the nomenclatural type strain of *S. vitulus*, DD 756, and the nomenclatural type strain of *S. sciuri*, DD 4277, as the reference strains. In these two-dimensional representations, the visual distinctions between the unique *S. vitulus* cluster and the other species tested are relatively clear. Clusters of points surrounding the reference strains are clearly identified by those reference strains.

Using the complete 512-dimensional-space analysis and the corresponding correlation values, we could clearly distinguish between *S. vitulus* and all of the other species tested. An organism could be identified as a member of *S. vitulus* by the squared correlation value of its pattern obtained from the 512-dimensional-space analysis, even if the pattern did not exactly match one of the pattern types associated with *S. vitulus*. In this study, squared pattern correlation values greater than 0.71 appeared to be strong evidence that strains belonged to the same species.

**Cell wall peptidoglycan and teichoic acid.** We also determined the peptidoglycan compositions of *S. vitulus* DD 756<sup>T</sup> (T = type strain), DD 771, and DD 4523, *S. lentus* DD 4202, *S. sciuri* DD 4277<sup>T</sup>, and *Staphylococcus aureus* DD 863<sup>T</sup>. The peptidoglycan of *S. vitulus* strains is type L-Lys-L-Ala-Gly<sub>3-3,4</sub>



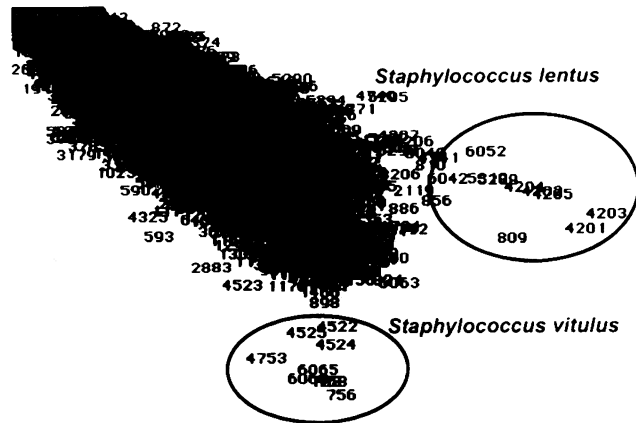


FIG. 3. Clustering of patterns as determined by transforming each pattern into a point in 512-dimensional space and depicting the points in a two-dimensional representation by using *S. lentus* DD 4203<sup>T</sup> and *S. vitulus* DD 756<sup>T</sup> to define the two dimensions. The strain number is placed in the appropriate location for each point on the figure. Data for approximately 5,550 strains belonging to 200 species are shown.

peptidoglycan. This peptidoglycan type is also present in *S. lentus* and *S. sciuri* (27).

The teichoic acids of *S. vitulus* are based on glycerol. Strain DD 756<sup>T</sup> has a glycerol teichoic acid with an integrated *N*-acetylglucosamine 1-phosphate; strain DD 771 has a glycerol teichoic acid substituted at a high level with *N*-acetylgalactosamine; and strain DD 4523 has a glycerol teichoic acid substituted at a low level with *N*-acetylglucosamine.

**DNA base composition.** The guanine-plus-cytosine content of *S. vitulus* DD 756<sup>T</sup> DNA is 34 mol%; the DNA guanine-plus-cytosine content of strains DD 771 and DD 4523 is 35 mol%; and the *S. sciuri* and *S. lentus* DNA guanine-plus-cytosine contents are between 30 and 36 mol% (20).

**Description of *Staphylococcus vitulus* sp. nov.** *Staphylococcus vitulus* (vit'u.lus. N. L. n. vitulus, veal). The description of *S. vitulus* below is based on the results of a study of 11 strains.

A total of 10 of the 11 strains produce colonies that are less than 3 mm in diameter when the organisms are grown aerobi-

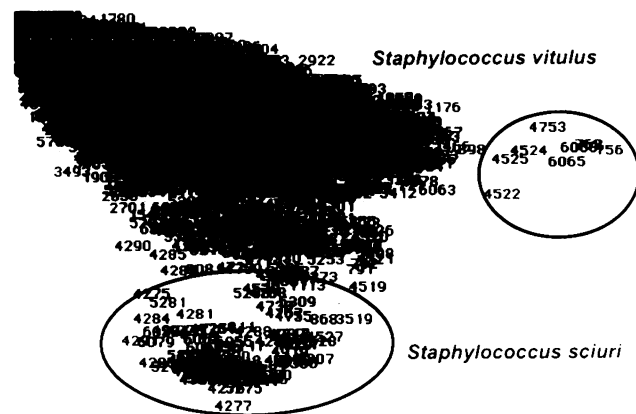


FIG. 4. Clustering of patterns as determined by transforming each pattern into a point in 512-dimensional space and depicting the points in a two-dimensional representation by using *S. vitulus* DD 756<sup>T</sup> and *S. sciuri* DD 4277<sup>T</sup> to define the two dimensions. The strain number is placed in the appropriate location for each point on the figure. Data for approximately 5,550 strains belonging to 200 species are shown.

TABLE 3. Variable characteristics of *S. vitulus* strains

Characteristic	No. of strains positive/ no. of strains tested	% of strains positive
Colony diam. of $\leq 3$ mm on P agar	10/11	91
Colony pigment	10/11	91
$\beta$ -Glucosidase activity <sup>a</sup>	9/11 <sup>b</sup>	82
Nitrate reduction	10/11	91
Esculin hydrolysis	7/11 (1/11) <sup>c</sup>	64 (9) <sup>c</sup>
Clumping factor:		
Standard slide test	0/11 (2/11)	0 (18)
Staph Latex kit (Remel) <sup>d</sup>	9/11 (2/11)	82 (18)
Acid produced aerobically from:		
D-Trehalose <sup>b</sup>	0/11 (4/11)	0 (36)
D-Xylose	7/11 (1/11)	64 (9)
D-Cellobiose	2/11 (1/11)	18 (9)
Salicin	1/11 (2/11)	9 (18)
D-Ribose	9/11 (2/11)	82 (18)

<sup>a</sup> Activity determined by the API STAPH-IDENT system.

<sup>b</sup> Most strains that produced a dd 756 pattern were positive for  $\beta$ -glucosidase activity and did not produce acid from mannose or trehalose (the only exception was weak acid production from trehalose by strain DD 6065).

<sup>c</sup> Weak positive reactions were not included as positive reactions. The values in parentheses are the number of strains that exhibited weak positive reactions/total number of strains tested and the percentage of strains that exhibited a weak positive reaction.

<sup>d</sup> The Staph Latex kit detected both clumping factor and protein A.

cally on P agar (18) at 35°C. Colonies on P agar are usually raised with ulcerated, irregular centers, are opaque, and often have sectored or irregular edges; 10 of the 11 strains studied produce cream to yellow pigmentation, and 1 strain produces unpigmented colonies. Colonies grown on tryptic soy agar are much larger (8 to 12 mm in diameter) and not as irregular as colonies grown on P agar.

Colony growth is reduced or inhibited at 40°C, and none of the strains grows at 45°C. The cells are not motile, and the organisms do not grow anaerobically in thioglycolate semisolid agar. However, they exhibit minimal growth on sheep blood agar and tryptic soy agar plates incubated at 35°C in anaerobic jars. The cells are gram-positive cocci, do not form spores, and occur singly, in pairs, in tetrads, and in clusters.

All strains produce zones of greening on bovine blood agar. All strains are negative for staphylocoagulase, thermonuclease, alkaline phosphatase, pyrrolidonyl arylamidase, ornithine decarboxylase, urease,  $\beta$ -glucuronidase, and  $\beta$ -galactosidase activities, arginine utilization, and acetoin production. All strains are negative for aerobic production of acid from the following substrates: mannose, lactose, galactose, melezitose, xylitol, rhamnose, turanose, arabinose, maltose, *N*-acetylglucosamine, and raffinose. All strains are negative for anaerobic production of acid from glucose and mannitol. All strains are positive for catalase activity, modified oxidase activity, and aerobic production of acid from the following substrates: mannitol, glycerol, sucrose, and fructose. The variable characteristics of *S. vitulus* are shown in Table 3.

The major API profiles are 4200 (six strains) and 4700 (three strains). STAPH Trac profiles exhibit variation with respect to acid production from maltose, raffinose, xylose, and trehalose, nitrate reduction, alkaline phosphatase, and acetoin production, indicating that there is no predominant profile.

**EcoRI fragments containing rRNA sequences.** The patterns of all *S. vitulus* strains cluster on the basis of similarity and are distinguishable from the patterns of all other bacteria examined, including all previously described *Staphylococcus* species. The species can be recognized by a high level of correlation with the pattern types described in this paper.

TABLE 4. Characteristics that differentiate *S. vitulus* from other novobiocin-resistant, oxidase-positive *Staphylococcus* species<sup>a</sup>

Characteristic	<i>S. vitulus</i>	<i>S. sciuri</i>	<i>S. lentus</i>
Colony size of $\geq 6$ mm on P agar	—	+	—
Anaerobic growth	—	(+)	(±)
Staph Latex kit reaction (Remel) <sup>b</sup>	+	d	d
Hemolysis of bovine blood	± <sup>c</sup>	—	—
Alkaline phosphatase activity	—	+	(±)
$\beta$ -Glucosidase activity	d	+	+
Esculin hydrolysis	d	+	+
Acid produced aerobically from:			
D-Trehalose	(d)	+	+
D-Mannose	—	(d)	(+)
D-Turanose	—	(±)	(±)
D-Xylose	(d)	(d)	(±)
D-Cellobiose	(d)	+	+
L-Arabinose	—	d	d
Maltose	—	(d)	d
N-acetyl-D-glucosamine	—	d	d
Raffinose	—	—	+

<sup>a</sup> +, 90% or more of the strains are positive; ±, 90% or more of the strains are weakly positive; —, 0 to 10% of the strains are positive; d, 11 to 89% of the strains are positive. Parentheses indicate that a response is delayed.

<sup>b</sup> The Staph Latex kit detected both clumping factor and protein A.

<sup>c</sup> *S. vitulus* produces greening during this test.

**Antibiotic susceptibilities.** As determined by agar disk diffusion tests, all strains of *S. vitulus* are susceptible to furazolidone, penicillin, erythromycin, and clindamycin. All strains are resistant to bacitracin and novobiocin. Three strains that produce pattern type dd 756 (DD 756<sup>T</sup>, DD 4524, and DD 4753) are resistant to tetracycline.

**Description of the type strain.** The type strain of *S. vitulus* is ATCC 51145 (= DD 756). It has all of the characteristics of the species described above. In addition, it has the properties described below.

Cells are spherical (diameter, 0.9 to 1.1  $\mu$ m) and occur singly, in pairs, in tetrads, and in clusters.

P agar colonies are raised with ulcerated, irregular centers, irregular edges, and cream pigmentation; the colonies are approximately 2 mm in diameter. Colonies on tryptic soy agar are raised with wide, depressed centers; each colony has a slightly irregular edge, and is pigmented, with a pinkish center blending to a cream edge. These colonies are approximately 10 to 11 mm in diameter.  $\beta$ -Glucosidase is produced. Nitrates are reduced to nitrites. Esculin is not hydrolyzed. The standard slide test is negative for clumping factor. The Staph Latex kit reaction is strongly positive.

Acid is produced aerobically from D-xylose and D-ribose. No acid is produced aerobically from D-cellobiose, salicin, and D-trehalose.

The type strain is resistant to tetracycline.

The guanine-plus-cytosine content of the DNA is 34 mol%.

The type strain produces a type dd 756 pattern when it is studied by using the methods described in this paper.

**Distinguishing characteristics.** *S. vitulus* can be distinguished from other bacterial species by examining the pattern of restriction fragments containing rRNA sequences that results when the methods described in this paper are used. *S. vitulus* can also be distinguished by its novobiocin resistance, positive oxidase reaction, strong positive Staph Latex kit reaction, absence of alkaline phosphatase activity, and lack of acid production from L-arabinose, maltose, N-acetylglucosamine, D-mannose, and raffinose. The major phenotypic

features that are useful for distinguishing *S. vitulus* from other novobiocin-resistant, oxidase-positive *Staphylococcus* species are summarized in Table 4.

#### ACKNOWLEDGMENTS

We thank Bill Bowen and V. Thayer (National Marine Fisheries, Beaufort, N.C.) for obtaining bacterial specimens from dolphins and a pilot whale and Herman Berkhoff (College of Veterinary Medicine, North Carolina State University, Raleigh) for assisting in obtaining bacterial specimens from dairy cattle and horses. We also thank Joe Neubauer and Channeary Iem (DuPont Company) for technical assistance, Lucy Ganfield and Dipti Schoop (DuPont Company) for preparing the probe and conjugate, and Charles Robertson and Robert Martin for modifying the Star One camera used.

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