Porphyromonas macacae comb. nov., a Consequence of *Bacteroides macacae* Being a Senior Synonym of *Porphyromonas salivosa*

DARIA N. LOVE*

Department of Veterinary Pathology, University of Sydney, New South Wales 2006 Australia

DNA-DNA hybridization studies were performed with members of the genus *Porphyromonas*. *Porphyromonas salivosa* NCTC 11632^T (T = type strain) exhibited an average level of intraspecies DNA-DNA hybridization with VPB 3313 and VPB 3444 of 95% and an average level of DNA-DNA hybridization with *Bacteroides macacae* ATCC 33141^T of 81%. However, while the cat strains and monkey strain which I studied exhibited sufficient hybridization to be considered members of a single species, the tight DNA hybridization clustering and phenotypic differences suggested that the biovars isolated from cats and monkeys are distinct and can be recognized by colonial and growth characteristics, lipase activity, sorbitol utilization, proteinase patterns, and whole-cell protein profiles on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels. As *B. macacae* is a senior synonym of *P. salivosa*, *Porphyromonas macacae* comb. nov. is proposed.

In 1987 Love et al. (10) described *Bacteroides salivus* as a pigmented asaccharolytic pathogen which was isolated from subcutaneous abscesses and pythoraxes of cats. The *B. salivus* strains exhibited little DNA-DNA hybridization with members of previously described pigmented asaccharolytic *Bacteroides* species. Also, original DNA-DNA hybridization experiments revealed that the levels of hybridization between feline strains and *Bacteroides macacae* ATCC 33141^T (T = type strain) (2, 17) were not significant. These findings and the reported fermentation of a limited number of sugars by *B. macacae* resulted in *B. macacae* not being included in the final studies in which *B. salivus* was described. In 1992, the latter species was reassigned to the genus *Porphyromonas* as *Porphyromonas salivosa* (9). However, since that time, workers in two laboratories (6, 13a) have reported that *P. salivosa* NCTC 11632^T ferments glucose, lactose, and sucrose in prereduced anaerobically sterilized media, and Paster et al. (15) have shown that *P. salivosa* NCTC 11632^T exhibits 99.3% rRNA sequence homology with *B. macacae* ATCC 33141^T. In this paper I describe the results of DNA relatedness studies performed with *P. salivosa* strains isolated from cats and of a comparison of these strains with *B. macacae* ATCC 33141^T (obtained from H. Jousimies-Somer). Below I propose that *B. macacae* should be assigned to the genus *Porphyromonas*, as *Porphyromonas macacae* comb. nov.

MATERIALS AND METHODS

Bacterial strains. All of the strains used in this study are shown in Table 1.

Growth conditions and biochemical methods. The growth conditions and biochemical methods used in this study have been described previously (1, 10–12). Cells for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) were grown anaerobically for 3 days on 5% defibrinated sheep blood agar plates (blood agar base no. 2; Oxoid, Ltd., Basingstoke, England) containing additional hemin and menadione, formate and fumarate (4), and proteose peptone (1.5%, wt/vol; Difco Laboratories, Detroit, Mich.) in the presence of a streak of *Staphylococcus epidermidis* to enhance growth and pigmentation. *P. salivosa* NCTC 11632^T and *B. macacae* ATCC 33141^T were grown on similar plates with and without a streak of *S. epidermidis* for comparisons of growth rate and colonial characteristics.

DNA hybridization. DNA was isolated essentially as described previously (13). Small amounts (5 µg) of fragmented and denatured DNA were labeled with ¹²⁵I

by using a variation of the thallium chloride method (16, 18). The S1 nuclease procedure was used to estimate percentages of hybridization as described by Johnson (5). Background reassociation values and the amounts of S1 nuclease-resistant material in the labeled preparations were determined after denatured unlabeled bacterial DNA was replaced with 50 µl (20 µg) of sheared native salmon sperm DNA. Results were recorded as the means of three determinations by using DNAs extracted from two different batches of cells on separate occasions; each DNA preparation was iodinated on two occasions.

SDS-PAGE of whole-cell proteins. Cells grown for 5 days were harvested from plates and washed in sterile water before lysis in sample buffer containing (final concentrations) 4% SDS, 20% glycerol, 100 mM dithiothreitol, and 125 mM Tris-HCl (pH 7.8). Preparations were electrophoresed on 12% acrylamide gels, and SDS-PAGE (7) was carried out with a Hoefer Mighty Small II apparatus (Hoefer Scientific Instruments, San Francisco, Calif.).

SDS-PAGE detection of proteinase activity. The modified method of North et al. (14) was used for SDS-PAGE detection of proteinase activity. Briefly, gelatin (final concentration, 0.05%, wt/vol) was added to 7.5% polyacrylamide, and polymerization was allowed to proceed normally. Washed *P. salivosa* NCTC 11632^T and *B. macacae* ATCC 33141^T cells harvested as described above after 5 days of growth on plates containing *S. epidermidis* were sonicated in a Cup Horn (three bursts [3 min each] at 60% duty cycle; output, 6) and a model W-375 cell disruptor (Heat Systems Ultrasonics, Inc.). Sucrose (final concentration, 10%) was added to crude cell extracts (which were clarified by centrifugation at 30,000 × g for 30 min at 4°C), and electrophoresis was carried out at room temperature at a constant voltage of 130 V until completion. The gel was gently washed in distilled water containing 2.5% (vol/vol) Triton X-100 for 1 h with four changes of solution before incubation overnight at 22°C in 10 ml of 100 mM Tris-HCl (pH 8). Cysteine was added to a final concentration of 50 mM (to ensure optimal reduction and activity of proteinases), and the gel was incubated for an additional 1.5 h before it was stained with 0.025% (wt/vol) Coomassie brilliant blue R-250 in 7.5% (vol/vol) acetic acid–10% (vol/vol) methanol. After 2 h, the gel was destained in 10% (vol/vol) acetic acid, and proteolytic activity was visualized as a clear band against a blue background.

RESULTS AND DISCUSSION

Colonies of *B. macacae* ATCC 33141^T were 0.1 to 0.2 mm in diameter, entire, and dome shaped after 6 days of incubation on blood agar plates. After 9 days in the presence of *S. epidermidis*, however, the colonies were 1.0 to 1.5 mm in diameter, entire, umbonate with a central depression, and creamy brown. As incubation progressed and where colonies were crowded, each colony surface was wrinkled with multiple central depressions and peripheral ridging. In contrast, after 6 days of incubation, cat strain NCTC 11641^T produced pinpoint colonies, but these colonies were consistently larger colonies than *B. macacae* ATCC 33141^T colonies. After 9 days in the presence of *S. epidermidis*, the NCTC 11641^T colonies were 1.0 mm in diameter, entire, high domed shaped, smooth, and creamy brown. The colonies did not become wrinkled or

* Phone: 61-2-692 2454. Fax: 61-2-552 6526. Electronic mail address: suvp00@angis.su.oz.au.

TABLE 1. Levels of DNA-DNA hybridization for *P. salivosa* and *P. macacae* comb. nov. with type strains of *Porphyromonas* species and members of the *Porphyromonas* rRNA cluster

Source of unlabeled DNA		% Hybridization with labeled DNA from ^a :	
Species or group	Strain	<i>P. salivosa</i> NCTC 11632 ^T	<i>P. macacae</i> ATCC 33141 ^T
<i>P. salivosa</i>	NCTC 11632 ^T	100	83
	VPB 3313	95	79
	VPB 3444	94	81
<i>P. macacae</i> comb. nov.	ATCC 33141 ^T	84	100
<i>P. gingivalis</i>	ATCC 33277 ^T	7	0
<i>P. gingivalis</i> cat strain	VPB 3492	23	5
<i>P. circumdentaria</i>	NCTC 12469 ^T	8	1
<i>P. canoris</i>	NCTC 12835 ^T	5	8
<i>P. endodontalis</i>	ATCC 35406 ^T	3	1
<i>P. asaccharolytica</i>	ATCC 25260 ^T	<1	3
<i>Bacteroides levii</i>	ATCC 29147 ^T	<1	4

^a The reference values were normalized to 100%.

display depressions after more incubation or where crowding occurred.

The whole-cell protein and proteinase profiles on SDS-PAGE gels for *B. macacae* ATCC 33142^T and *P. salivosa* are shown in Fig. 1.

My DNA-DNA hybridization results (Table 1) showed that cat strains NCTC 11632^T, VPB 3313, and VPB 3444 exhibited negligible levels of hybridization with all of the other strains tested except the *B. macacae* strain. As the level of DNA-DNA hybridization was sufficient to consider *B. macacae* and *P. salivosa* members of the same species, *P. salivosa* is a later synonym of *B. macacae*.

My hybridization data, the findings of Paster et al. (15) that *B. macacae* ATCC 33141^T and *P. salivosa* NCTC 11632^T exhibited 99.3% rRNA homology and were deeply embedded

in the *Porphyromonas* rRNA cluster, and the presence of 13-methyltetradecanoic acid as a major cellular fatty acid (8; unpublished data) provide compelling evidence that *B. macacae* ATCC 33141^T should be transferred to the genus *Porphyromonas* as *Porphyromonas macacae* comb. nov.

An emended generalized description of this taxon based on my data and data in previous reports (1, 2, 9, 10, 17) is given below.

Emended description of *Porphyromonas macacae* comb. nov.

Cells are anaerobic, nonsporing, nonmotile, gram-negative rods or coccoid forms. After 6 days on blood agar plates, colonies of *P. macacae* ATCC 33141^T are 0.1 to 0.2 mm in diameter, entire, and dome shaped. After 9 days in the presence of *S. epidermidis*, however, the colonies are 1.0 to 1.5 mm in diameter, entire, umbonate with a central depression, and creamy brown. As incubation progresses and where colonies are crowded, the surfaces of colonies may become wrinkled with multiple central depressions and peripheral ridging. Colonies do not fluoresce under UV light (265 and 366 nm), and vitamin K and hemin are required for growth. Carbohydrate fermentation is not detected with adonitol, cellobiose, fructose, glycogen, inositol, lactose, maltose, mannitol, rhamnose, salicin, starch, trehalose, or xylose, but fermentation of glucose, sucrose, sorbitol, galactose, and mannose usually occurs. Cells produce major amounts (>10 µmol/ml) of acetate, butyrate, and phenylacetate, and minor amounts (<10 µmol/ml) of isobutyrate and isovalerate are detected in cooked meat medium. Cells are catalase positive, liquefy gelatin, and have trypsin-like activity but do not produce lipase. *P. macacae* ATCC 33141^T contains 13-methyltetradecanoic acid (iso-C_{15:0} acid) as a major cellular fatty acid, and malate, glutamate, glucose-6-phosphate, and 6-phosphogluconate dehydrogenases are present. Strains have been isolated from oral cavities, subcutaneous abscesses, and pyothoraxes of animals, including cats and monkeys. *P. macacae* ATCC 33141^T exhibits an average level of DNA-DNA hybridization with strains isolated from cats of 81% but no significant hybridization with other members of the genus *Porphyromonas*.

Consistent with the tight clustering of DNA-DNA hybridization values, *P. macacae* ATCC 33141^T can be distinguished by phenotypic criteria from cat strains, suggesting that cat and monkey biovars exist. Members of cat and monkey biovars have different colonial morphologies on blood agar and produce different whole-cell protein and proteinase profiles on SDS-PAGE gels (Fig. 1). In addition, the cat biovar is lipase positive and produces glutamyl-glutamic arylamidase (3) but does not ferment sorbitol.

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FIG. 1. (A) SDS-7.5% PAGE gel containing 0.05% gelatin stained with Coomassie blue after development for proteinase activity. Lane 1, molecular mass (kilodaltons) indicates area of activity of proteinases; lane 2, *P. salivosa* NCTC 11632^T; lane 3, *P. macacae* ATCC 33141^T. (B) SDS-12% PAGE gel of whole-cell proteins denatured in sample buffer (4% SDS, 20% glycerol, 100 mM dithiothreitol, 125 mM Tris-HCl [pH 6.8]) and stained with Coomassie blue. Lane 4, *P. salivosa* NCTC 11632^T; lane 5, *P. macacae* ATCC 33141^T; lane 6, molecular mass markers (in kilodaltons).

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