

Classification of *Proteus vulgaris* biogroup 3 with recognition of *Proteus hauseri* sp. nov., nom. rev. and unnamed *Proteus* genomospecies 4, 5 and 6

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Strains traditionally identified as *Proteus vulgaris* formed three biogroups. Biogroup 1, characterized by negative reactions for indole production, salicin fermentation and aesculin hydrolysis, is now known as *Proteus penneri*. Biogroup 2, characterized by positive reactions for indole, salicin and aesculin, was shown by DNA hybridization (hydroxyapatite method) to be a genetic species separate from biogroup 1 and from biogroup 3 which is positive for indole production and negative for salicin and aesculin. In this study, 52 strains were examined, of which 36 strains were *Proteus vulgaris* biogroup 3, which included the current type strain of the species *P. vulgaris* (ATCC 29905^T), and compared to seven strains of *Proteus vulgaris* biogroup 2 and nine type strains of other species in the genera *Proteus*, *Providencia* and *Morganella*. By DNA hybridization, these 36 strains were separated into four distinct groups, designated as *Proteus* genomospecies 3, 4, 5 and 6. DNAs within each separate *Proteus* genomospecies were 74–99% related to each other in 60 °C hybridization reactions with ≤4.5% divergence between related sequences. *Proteus* genomospecies 3 contained the former *P. vulgaris* type strain and one other strain and was negative in reactions for salicin fermentation, aesculin hydrolysis and deoxyribonuclease, unlike the reactions associated with strains considered as typical *P. vulgaris* which are positive in reactions for salicin, aesculin and DNase. Genomospecies 3 can be distinguished from *Proteus* genomospecies 4, 5 and 6 because it is negative for Jordan's tartrate. *Proteus* genomospecies 4, containing five strains, was differentiated from *Proteus penneri*, genomospecies 3 and 6 and most, but not all, strains of genomospecies 5, by its ability to ferment L-rhamnose. *Proteus* genomospecies 5 and 6, containing 18 and 11 strains, respectively, could not be separated from each other by traditional biochemical tests, by carbon source utilization tests or SDS-PAGE of whole-cell proteins. In an earlier publication, a request was made to the Judicial Commission that the former type strain of *P. vulgaris* (ATCC 13315) be replaced by *P. vulgaris* biogroup 2 strain ATCC 29905^T, a strain considered more biochemically typical of *P. vulgaris* strains. This would have the effect of assigning the name *P. vulgaris* to *P. vulgaris* biogroup 2. Since this request has been acceded to, the name *Proteus hauseri* is herein proposed for *Proteus vulgaris* genomospecies 3. Its type strain is ATCC 700826^T. *Proteus* genomospecies 4, 5 and 6 will remain unnamed until better phenotypic differentiation can be accomplished. All *Proteus* genomospecies were similar in their antimicrobial susceptibility patterns. Nineteen strains were isolated from urine, four from faeces, two from wounds, nine from other human sources and two from animals.

Keywords: *Proteus vulgaris*, *Proteus hauseri*

INTRODUCTION

Much has been written about the taxonomy of the genus *Proteus* since the original publication by Hauser (1885) that established the genus. The genus originally had four species: *Proteus mirabilis*, *Proteus rettgeri*, *Proteus morganii* and *Proteus vulgaris* which is the type species. The genus is a frequent cause of urinary tract infections, but is not usually a nosocomial pathogen. Brenner *et al.* (1978) showed by DNA–DNA hybridi-

zation that *P. vulgaris* was a heterogeneous group with at least three biogroups. In 1982, *P. vulgaris* biogroup 1 (= genomospecies 1) was named *Proteus penneri* (Hickman *et al.*, 1982) and was distinguished by its negative reactions for indole production, salicin fermentation and aesculin hydrolysis. The remaining two biogroups were both positive for indole production. However, biogroup 2 (= genomospecies 2) was positive for salicin and aesculin and biogroup 3 was negative for salicin and aesculin. The DNA

Table 1. Bacterial strains used in this study

CDC strain no. (additional strain designations)	Location of sender	Source of specimen
PR 1 ^T (ATCC 29905 ^T = CDC 9166-79 ^T = NCTC 13145 ^T = CCUG 35382 ^T = CIP 104989 ^T)	Denmark	Stool (?)
PR 3	Denmark	Unknown
PR 10	Denmark	Unknown
PR 124 (CDC 4138-92)	Unknown	Unknown
1086-80 (ATCC 13315 = CDC 9079-77 = CDC 2130-74 = NCTC 4175 = CCUG 6327 = CIP 58.60 = DSM 30118)	Unknown	Unknown
1822-62	Massachusetts	Human, unknown
5272-68 (ATCC 27972)	New Jersey	Abdominal wound
1070-73	Iowa	Neck lesion
1608-73	Delaware	Unknown
2139-74	South Dakota	Urine
1425-75	Washington	Sputum
0707-76	Arizona	Urine
1944-77	Canada	Sputum
2049-79	Arizona	Urine
1732-80 ^T (ATCC 700826 ^T = CCUG 35386 ^T)	Tennessee	Human, unknown
1392-81	Canada	Urine
1393-81	Canada	Urine
1394-81	Canada	Urine
1395-81	Canada	Urine
1396-81	Canada	Urine
1397-81	Canada	Stool
1398-81	Canada	Urine
1399-81	Canada	Urine
1400-81	Canada	Urine
1401-81	Canada	Stool
1402-81	Canada	Stool
1404-81 (ATCC 51470 = CCUG 35385)	Canada	Urine
1405-81	Canada	Urine
1406-81	Canada	Urine
1407-81	Canada	Stool
1408-81	Canada	Urine
1409-81	Canada	Sputum
1410-81	Canada	Urine
1411-81	Canada	Urine
4513-89	Colorado	Urine
8390-93 (ATCC 51471 = 87B = CCUG 35381)	Great Britain	Urine
8391-93 (107B)	Great Britain	Wound
8385-93 (ATCC 51469 = 111B = CCUG 35384)	Great Britain	Urine
8386-93 (119B)	Great Britain	Urine
8388-93 (123B)	Great Britain	Urine
8387-93 (PS 53)	Great Britain	Animal bedding
8389-93 (PS 36)	Great Britain	Animal bedding
GBL 1561	Wisconsin	Human, unknown

Table 2. DNA relatedness of 36 *P. vulgaris* biogroup 3 strains

Source of unlabelled DNA	Relatedness (%) to labelled DNA from:															
	<i>P. vulgaris</i> PR 1 ^T				<i>P. hauseri</i> 1732-80 ^T				<i>Proteus</i> genomospecies 4 8385-93 (111B)				<i>Proteus</i> genomospecies 5 1404-81			
	60 °C	SEM	D*	75 °C	60 °C	SEM	D	75 °C	60 °C	SEM	D	75 °C	60 °C	SEM	D	75 °C
<i>Proteus vulgaris</i> (biogroup 2)																
PR 1 ^T (ATCC 29905 ^T)	100	0-0	100		57	3-5		15	62	3-5	3-5	39	70	1-5		39
PR 3	100	0	0-0	98												
1608-73	99	0-9	0-0	97												
PR 10	96	2-1	0-0	100												
1944-77	91	2-8	1-0	78												
1425-75	80	2-1	0-0	78												
2049-79	64	2-2	0-0	70												
<i>Proteus hauseri</i> 1732-80 ^T (ATCC 700826 ^T)					92	3-9	0-5	100								
1086-80 (ATCC 13315)	44	1-0	11-0	13	100		0-0	100					38	0-0	9-5	
<i>Proteus</i> genomospecies 4 8385-93 (ATCC 51469)					55	8-5			100	—	0-0	100	63	2-1	6-5	30
1070-73	68	0		35	68	3-5		19	97	3-0	0-0	93	72	3-0	5-5	45
PR 124					42	2-0			94	4-0	0-0	94	68	3-5	6-0	34
8386-93					61	6-5			83	0	0-0	87	71	2-5	5-5	38
8387-93					50	4-5			82	4-6	3-0	80	52	1-5	9-0	23
<i>Proteus</i> genomospecies 5 1404-81 (ATCC 51470)					42	2-0			65	4-5	6-5	38	100	—	0-0	100
1394-81					44	2-0							90	4-5	0-5	87
1408-81					6	4-0							89	1-0	2-5	83
1406-81					28	1-0			62	5-1	5-5	37	89	4-2	3-0	64
1411-81					59	8-0							87	1-0	2-0	83
1407-81					17	1-0			64	5-2	4-5	46	87	0	3-5	65
8388-93					47	2-0			57	0-5	5-0	43	87	0-5	4-0	62
2139-74	60	0-3	7-0	28	43	1-0		17	60	4-0	5-0	50	86	2-3	2-0	77
1401-81					49	5-0			85	2-0	0-5	83				
1410-81	54	1-0		30	21	1-0			56	1-0	5-0	47	85	2-0	3-0	68
1822-62					66	1-5			69	3-3	4-5	35	85	4-2	3-5	61
1409-81					26	1-5			58	4-0	8-5	37	82	3-6	4-0	58
1392-81					35	1-5		15	60	2-5	5-5	48	81	3-2	3-0	70
0707-76	50	0-7	6-5	25	34	4-0		14	59	3-0	5-0	49	80	7-0	1-5	66
1402-81					46	1-0			51	3-0	6-0	43	80	3-4	2-0	72
8389-93					63	1-5			57	1-0	6-0	38	80	3-5	3-5	72
1399-81					46	7-5			54	5-0	6-0	46	77	3-0	3-0	63
1400-81					19	1-0			44	3-5	6-5	32	74	3-4	2-5	62
<i>Proteus</i> genomospecies 6 8390-93 (ATCC 51471)					61	2-5			47	2-0	6-0	35	73	2-5	6-0	50
GBL 1561					60	2-0			62	3-5	5-5	35	70	4-8	6-5	35
5272-68					56	4-0			60	4-5	4-5	37	73	4-5	3-5	48
4513-89					41	2-0			47	3-5	6-0	38	68	4-0	8-5	35
1396-81					48	1-5			44	5-0	6-5	36	68	2-7	4-5	42
1393-81									59	0-5	6-5	40	75	3-0	4-0	52
1398-81					22	0-5		8	54	0-5	7-0	38	71	3-8	5-0	40
1405-81					46	0			45	4-0	6-5	35	66	5-5	4-5	28
1395-81					33	2-0			29	3-5	8-0	20	61	3-3	7-5	30
8391-93					59	2-5			54	3-0	5-0	40	78	3-1	4-5	42
1397-81					51	0-5			53	0-5	6-0	37	73	2-5	4-0	47
<i>Proteus penneri</i> 1808-73 ^T	54	0-6	7-5	30	60	1-5			67	8-0	2-5	41	70	4-2	6-0	37
<i>Proteus mirabilis</i> PR 14 ^T					50	3-0							46	4-5		
<i>Proteus myxofaciens</i> ATCC 19692 ^T					41	0							38	3-0		
<i>Providencia rustigianii</i> 132-68 ^T					25	1-5							19	1-0		
<i>Providencia alcalifaciens</i> 3370-67 ^T					20	0							18	2-0		
<i>Providencia heimbachae</i> ATCC 35613 ^T													17	1-0		2
<i>Providencia rettgeri</i> 1163 ^T					17	2-0							17	1-0		
<i>Morganella morganii</i> subsp. <i>morganii</i> 4567-84 ^T					24	0							16	2-0		
<i>Providencia stuartii</i> 2896-68 ^T					16	0-5							14	0-5		

* D, Divergence (%).

hybridization reference strain of *P. vulgaris* (PR 1^T = ATCC 29905^T) genetically belongs in and has the phenotypic characteristics of biogroup 2 in that it is positive in tests for indole, salicin and aesculin. In a previous publication (Brenner, 1995) this strain was proposed as the neotype strain of *P. vulgaris*. The former type strain of *P. vulgaris*, however, belongs to biogroup 3 by DNA–DNA hybridization and is very uncharacteristic of biogroup 2 in its biochemical reactions. It belongs to biogroup 3 phenotypically because it is positive for indole production and is negative for salicin and aesculin. In taxonomic studies, McKell & Jones (1976) reported that this strain was clearly atypical and fell outside both *P. vulgaris* subclusters. In studies by Costas *et al.* (1993), utilizing SDS-PAGE protein patterns, the type strain belonged to a separate small subcluster. In this report, we further define biogroup 3.

METHODS

Bacterial strains. Included in the study were 7 strains of *Proteus vulgaris* biogroup 2, 36 strains of *Proteus vulgaris* biogroup 3 and 9 type and reference hybridization strains representing most of the species in the genera *Proteus*, *Providencia* and *Morganella* (Table 1). All the strains were maintained in defibrinated sheep blood and stored frozen at –70 °C. They were passed twice on Trypticase Soy Agar with 5% sheep blood (TSA II: Becton Dickinson) before use.

Media and biochemical tests. The biochemical tests were performed on conventional media as previously described (Farmer *et al.*, 1980), with some modifications by Hickman & Farmer (1978). Incubations were at 35 °C and test results were read at 24 h, 48 h and 7 d, unless otherwise noted. Commercial media were used whenever possible. Carbon source utilization tests were done at the Institut Pasteur using Biotype 100 strips (bioMérieux) that contained 99 pure carbon sources. The strips were inoculated using Biotype medium 1, according to the manufacturer's instructions.

Antimicrobial susceptibilities. MIC tests were performed by the broth microdilution method as described by the National Committee for Clinical Laboratory Standards (1997) using Mueller–Hinton broth (BDMS). Quality control organisms included *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853.

DNA methods. The preparation, isolation and purification of labelled and unlabelled DNA, the method used for DNA reassociation and the method used to separate single- and double-stranded DNA on hydroxyapatite have been described by Brenner *et al.* (1982, 1993). DNAs were labelled enzymically *in vitro* with [³²P]dCTP using a nick translation reagent kit (Bethesda Research Laboratories) as directed by the manufacturer.

Electrophoretic protein patterns. The preparation of protein samples, electrophoresis, staining and scanning of gels as well as the analysis and computation of similarity of patterns have been described by Costas *et al.* (1993).

RESULTS AND DISCUSSION

This study determined that biogroup 3 is actually composed of four distinct DNA groups that were

designated *Proteus* genomospecies 3, 4, 5 and 6 (Table 2). The former *P. vulgaris* type strain (ATCC 13315) and only one other strain belong to *Proteus* genomospecies 3. Because of the association of the specific epithet *P. vulgaris* with the former type strain ATCC 13315 (Buchanan *et al.*, 1963), which is not typical of the majority of strains ascribed to this species, a Request for an Opinion was made to the Judicial Commission of the International Committee on Systematic Bacteriology for resolution of this problem (Brenner *et al.*, 1995). On the basis of DNA hybridization, Brenner *et al.* (1995) recommended that biogroup 2, which is commonly recognized in clinical laboratories, retain the name *Proteus vulgaris* and that the DNA hybridization reference strain PR 1^T (ATCC 29905^T) be designated as the neotype strain of the species. *P. vulgaris* strain PR 1^T has the characteristic biochemical reactions associated with biogroup 2. In 1999 that request was granted (Trüper, 1999).

Table 3 presents the reactions of three named species and three unnamed genomospecies of *Proteus*. *Proteus* genomospecies 4 can be separated phenotypically from *Proteus* genomospecies 3 by its positive tests for L-rhamnose fermentation, lipase production, Jordan's tartrate and DNase. *Proteus* genomospecies 4 can be separated from *Proteus* genomospecies 6 by its positive reaction for L-rhamnose, but cannot be differentiated from *Proteus* genomospecies 5 because there are three L-rhamnose-positive strains in the genomospecies 5 group. There are no definitive criteria for the phenotypic separation of *Proteus* genomospecies 4 and 5 or of genomospecies 5 and 6.

Of the 11 isolates of *Proteus* genomospecies 6 for which DNA relatedness was determined, 10 were negative in tests for salicin and aesculin. One strain (8391-93) with high reassociation constants was positive for salicin and aesculin, which would place it in biogroup 2. Repeat hybridization of this single strain against both 8390-93 (candidate type strain for *Proteus* genomospecies 6) and PR 1^T (ATCC 29905^T, neotype strain of *P. vulgaris*) yielded the same results, confirming its inclusion in *Proteus* genomospecies 6. However, this strain was negative in the test for DNase, unlike the strains of biogroup 2. With respect to the abnormally low relatedness between *P. hauseri* and one or two strains in genomospecies 5 and 6, there is no doubt as to which genomospecies these strains belong. Hence, these strains were not rehybridized.

When the 36 strains of biogroup 3 were tested in the Biotype 100 carbon source utilization strips, there were insufficient differences to allow differentiation of all four *Proteus* genomospecies. The exception was the utilization of L-rhamnose by all of the *Proteus* genomospecies 4 strains which correlated with the reactions obtained with Andrade's fermentation medium containing 0.5% L-rhamnose. Differentiation using L-rhamnose was complicated by the fact that three isolates of *Proteus* genomospecies 5 were L-rhamnose-positive using Andrade's medium and one isolate was L-rhamnose-positive in the carbon source strip.

Table 3. Biochemical characteristics of *Proteus penneri*, *Proteus vulgaris*, *Proteus hauseri* and *Proteus* genomospecies 4, 5 and 6

All taxa are positive in reactions for phenylalanine deaminase, tyrosine utilization and the production of acid from D-glucose and D-xylose. All taxa are negative in reactions for Voges–Proskauer, lysine and ornithine decarboxylase, arginine dihydrolase, malonate utilization, production of yellow pigment at 25 °C and acid production from D-adonitol, L-arabinose, D-arabitol, cellobiose, dulcitol, erythritol, *myo*-inositol, D-mannitol, D-mannose, melibiose, D-sorbitol and mucate. Boxed areas represent biochemical characteristics (percentage positive at 48 h) useful in differentiating *Proteus* species and genomospecies.

Test	<i>Proteus penneri</i> (54)*	<i>Proteus vulgaris</i> (7)	<i>Proteus hauseri</i> (2)	<i>Proteus</i> genomospecies 4 (5)	<i>Proteus</i> genomospecies 5 (18)	<i>Proteus</i> genomospecies 6 (11)
Indole production	0	100	100	100	100	100
Methyl red	100	86	100	100	100	100
Citrate (Simmons)	4	29	0	0	0	0
Hydrogen sulfide (on TSI Agar)	32	57	50 (–†)	80	94	73
Urea (Christensen)	98	86	100	100	100	100
Motility	89	57	100	100	94	100
Gelatin hydrolysis (22 °C)	56	57	100	100	100	100
Growth in KCN	98	100	100	100	100	90
D-Glucose						
Gas production	46	86	0	80	83	91
Acid production from:						
D-Galactose	96	83	100	100	100	100
Glycerol	40	29	0	0	0	0
Lactose	9	0	0	0	0	0
Maltose	96	100	100	100	100	100
α -Methyl-D-glucoside	81	86	50 (–)	60	0	10
Raffinose	9	0	0	0	0	0
L-Rhamnose	0	0	0	100	17	0
Salicin	0	100	0	0	0	9
Sucrose	96	100	100	100	100	100
Trehalose	62	0	0	20	12	20
Tartrate (Jordan)	89	14	0	100	100	100
Aesculin hydrolysis	0	100	0	0	0	9
Acetate utilization	12	14	0	0	12	18
Lipase (corn oil)	35	14	0	100	100	90
DNase (25 °C)	12	100	0	100	100	55
NO ₃ [–] →NO ₂ [–]	80	57	100	100	100	91
ONPG	10	0	0	0	0	0
Hydrogen sulfide (PIA)	47	57	50 (–)	80	94	80

* Number of strains in database.

† Reaction of type strain ATCC 700826^T (CDC 1732–80^T).

When the strains of biogroup 3 (representing *Proteus* genomospecies 3, 4, 5 and 6), in addition to those of the previous study, were included in the analysis of Costas *et al.* (1993), the original differentiation into clusters 3a and 3b was no longer apparent. A greater degree of heterogeneity was evident in the protein patterns of the biogroup 3 strains than in the other taxa examined, but there was no correlation with the four groups recognized by DNA–DNA hybridization.

We propose that *Proteus* genomospecies 3 become known as *Proteus hauseri*. *Proteus* genomospecies 3 contains only the original type strain of *P. vulgaris* and one other strain. We propose ATCC 700826^T (CDC

1732–80^T) as the type strain to avoid the possible confusion if ATCC 13315 (former type strain of *P. vulgaris*) were proposed as the *P. hauseri* type strain.

Proteus genomospecies 4, 5 and 6 have not been formally named since they cannot be phenotypically separated with certainty.

The MICs of 14 antimicrobial agents for isolates from *P. hauseri* and *Proteus* genomospecies 4, 5 and 6 are shown in Table 4. They were similar in their antimicrobial susceptibility patterns, being susceptible to amikacin, aztreonam, ceftazidime, ciprofloxacin, gentamicin, imipenem, mezlocillin, tobramycin and trimethoprim/sulfamethoxazole. They were resistant to

Table 4. Antimicrobial susceptibilities ($\mu\text{g ml}^{-1}$) of *Proteus hauseri* and *Proteus* genomospecies 4, 5 and 6

Antimicrobial	<i>Proteus hauseri</i>	<i>Proteus</i> genomospecies 4	<i>Proteus</i> genomospecies 5	<i>Proteus</i> genomospecies 6
Ampicillin	2–128	> 256	≥ 32	> 256
Mezlocillin	≤ 8	≤ 8	≤ 8 –16	≤ 4 –> 128
Aztreonam	≤ 1 –4	≤ 1	≤ 1	≤ 1
Imipenem	≤ 0.25 –1	2–16	0.5–4	≤ 0.25 –4
Tetracycline	≤ 0.5	≥ 16	1–> 32	1–> 32
Cefazolin	≤ 0.5 – ≥ 32	> 32	> 32	> 32
Cefoxitin	≤ 2	4	2–16	2–4
Cefotaxime	≤ 4	≤ 4	≤ 8	≤ 4
Ceftazidime	≤ 2	≤ 0.5	≤ 2	≤ 1
Trimethoprim/sulfamethoxazole (1:19)	≤ 0.12	≤ 0.25	≤ 0.12 –8	≤ 0.12 –> 16
Ciprofloxacin	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06
Amikacin	≤ 1	≤ 8	≤ 16	≤ 8
Gentamicin	≤ 0.25	≤ 4	1–8	≤ 4
Tobramycin	≤ 0.25	≤ 2	0.5–8	≤ 2

ampicillin and cefazolin and 63% were resistant to tetracycline. With cefotaxime, most strains were resistant by MIC tests. There were no distinct patterns of resistance that could be discerned among these four groups. The small numbers of strains in each group preclude us from drawing any conclusions about what may be useful differences in antibiograms among the groups. Caution must be exercised in translating the categorical interpretation of the broth dilution results to disk diffusion as discrepancies between the interpretative categories of two test methods have been noted for other *Proteus* and *Morganella* strains and some cephalosporins (Biedenbach & Jones, 1994).

Description of *Proteus hauseri* sp. nov., nom. rev.

Proteus hauseri (hau'ser.i. N.L. gen. n. *hauseri* to honour Gustav Hauser, the German microbiologist, who proposed the genus *Proteus* in 1885).

Corresponds to *Proteus* genomospecies 3. Strains are Gram-negative, oxidase-negative, fermentative, non-pigmented rods with the general characteristics of the family *Enterobacteriaceae* and of the genus *Proteus* (Table 3). The strains are positive for indole production and negative for aesculin hydrolysis and salicin fermentation. Biochemically these strains are similar to those commonly identified as *Proteus vulgaris*. They can be separated from the other *Proteus* genomospecies using L-rhamnose fermentation, DNase, lipase production and Jordan's tartrate utilization. Full biochemical reactions are given in Table 3 (useful biochemical reactions for the differentiation of the named species and unnamed genomospecies are boxed). Pathogenicity in humans and animals is undetermined. *P. hauseri* contains two strains from unknown sources. The type strain is ATCC 700826^T (CDC 1732-80^T).

Description of *Proteus* genomospecies 4

Proteus genomospecies 4 is generally separated from the other *Proteus* species because it ferments L-rhamnose. However, the strains can be separated from *Proteus* genomospecies 3 by positive reactions for DNase, lipase and Jordan's tartrate. Differentiation from *Proteus* genomospecies 5 may be difficult in the absence of a negative test for L-rhamnose. Two strains of this organism were isolated from human urine and one each from a neck wound and animal bedding. The source of one strain is unknown. Pathogenicity for humans and animals is undetermined. The candidate type strain is ATCC 51469^T (= CDC 8385-93^T = Hawkey 111B), isolated from a mid-stream urine sample (Bristol, Avon, UK).

Description of *Proteus* genomospecies 5

Positive for the production of lipase, DNase and utilization of Jordan's tartrate. The 18 strains of this organism were isolated from human urine (12), stool (3), sputum (1), animal bedding (1) and unknown (1). Pathogenicity for humans and animals is undetermined. The candidate type strain is ATCC 51470^T (= CDC 1404-81^T), isolated from a mid-stream urine sample (Toronto, Ontario, Canada).

Description of *Proteus* genomospecies 6

Biochemically similar to *Proteus* genomospecies 5 when it is tested against the substrates described herein. It is L-rhamnose-negative, but one strain is positive in tests for salicin and aesculin which would place it in biogroup 2. Unlike biogroup 2, however, it is DNase-negative. The 11 strains of this organism were isolated from human urine (7), wound (2), stool (1) and one

strain from an unknown human source. Pathogenicity for humans and animals is undetermined. The candidate type strain is ATCC 51471^T (= CDC 8390-93^T = Hawkey 87B) which was isolated from a mid-stream urine sample of a patient in Bristol Children's Hospital, Bristol, Avon, UK.

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REFERENCES

- Biedenbach, D. J. & Jones, R. N. (1994). Predictive accuracy of disk diffusion test for *Proteus vulgaris* and *Providencia* species against five newer orally administered cephalosporins, cefdinir, cefetamet, cefprozil, cefuroxime and loracarbef, *J Clin Microbiol* **32**, 559–562.
- Brenner, D. J., Farmer, J. J., III, Fanning, G. R., Steigerwalt, A. G., Klykken, P., Wathen, H. G., Hickman, F. W. & Ewing, W. H. (1978). Deoxyribonucleic acid relatedness of *Proteus* and *Providencia* species, *Int J Syst Bacteriol* **28**, 269–282.
- Brenner, D. J., McWhorter, A. C., Leete-Knutson, J. K. & Steigerwalt, A. G. (1982). *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds, *J Clin Microbiol* **15**, 1133–1140.
- Brenner, D. J., Grimont, P. A. D., Steigerwalt, A. G., Fanning, G. R., Ageron, E. & Riddle, C. F. (1993). Classification of citrobacteria by DNA hybridization: designation of *Citrobacter farmeri* sp. nov., *Citrobacter youngae* sp. nov., *Citrobacter braakii* sp. nov., *Citrobacter werkmanii* sp. nov., *Citrobacter sedlakii* sp. nov., and three unnamed *Citrobacter* genomospecies, *Int J Syst Bacteriol* **43**, 645–658.
- Brenner, D. J., Hickman-Brenner, F. W., Holmes, B., Hawkey, P. M., Penner, J. L., Grimont, P. A. D. & O'Hara, C. M. (1995). Replacement of NCTC 4175, the current type strain of *Proteus vulgaris*, with ATCC 29905: request for an opinion, *Int J Syst Bacteriol* **45**, 870–871.
- Buchanan, R. E., Seeliger, H. P. R. & Clark, W. A. (1963). Opinion 26. Designation of neotype strains (cultures) of type species of the bacterial genera *Salmonella*, *Shigella*, *Arizona*, *Escherichia*, *Citrobacter* and *Proteus* of the family *Enterobacteriaceae*, *Int Bull Bacteriol Nomencl Taxon* **13**, 35–36.
- Costas, M., Holmes, B., Frith, K. A., Riddle, C. & Hawkey, P. M. (1993). Identification and typing of *Proteus penneri* and *Proteus vulgaris* biogroups 2 and 3, from clinical sources, by computerized analysis of electrophoretic protein patterns, *J Appl Bacteriol* **75**, 489–498.
- Farmer, J. J., III, Asbury, M. A., Hickman, F. W., Brenner, D. J. & the *Enterobacteriaceae* Study Group (1980). *Enterobacter sakazakii*: a new species of 'Enterobacteriaceae' isolated from clinical specimens. *Int J Syst Bacteriol* **30**, 569–584.
- Hauser, G. (1885). Über Fäulnisbakterien und deren Beziehungen zur Septicämie. In *Ein Beitrag zur Morphologie der Spaltpilze*. p. 12. Leipzig: Vogel.
- Hickman, F. W. & Farmer, J. J., III (1978). *Salmonella typhi*: identification, antibiograms, serology, and bacteriophage typing, *Am J Med Technol* **44**, 1149–1159.
- Hickman, F. W., Steigerwalt, A. G., Farmer, J. J., III & Brenner, D. J. (1982). Identification of *Proteus penneri* sp. nov., formerly known as *Proteus vulgaris* indole negative or as *Proteus vulgaris* biogroup 1, *J Clin Microbiol* **15**, 1097–1102.
- McKell, J. & Jones, D. (1976). A numerical taxonomic study of *Proteus-Providencia* bacteria, *J Appl Bacteriol* **41**, 133–161.
- National Committee for Clinical Laboratory Standards (1997). Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. Wayne, PA: National Committee for Clinical Laboratory Standards.
- Trüper, H. G. (1999). Replacement of strain NCTC 4175, since 1963 the neotype strain of *Proteus vulgaris*, with strain ATCC 29905 – Opinion 70, *Int J Syst Bacteriol* **49**, 1949.