

## CASE REPORTS

### Isolation of *Buttiauxella gaviniae* from a Spinal Cord Patient with Urinary Bladder Pathology

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A gram-negative *Buttiauxella gaviniae*-like organism (LBV449) was isolated from a urine sample of a patient suffering from urine bladder pathology and neurological problems. The isolate was positive for adonitol fermentation and L-arginine dihydrolase and negative for melibiose and L-ornithine decarboxylase. The API 20E code was 3004113. Retrospectively, another isolate (ENT107), from a leg wound, was recovered from our collections and was shown to have similar biochemical characteristics. DNA-DNA hybridization showed 77% similarity between both strains, and strain LBV449 revealed 74% DNA-DNA similarity to the type strain of *B. gaviniae*. Neither 16S rRNA gene sequencing nor fatty acid analysis were useful for identification. The characteristic tRNA-PCR patterns obtained for these two clinical isolates consisted of fragments with lengths of 102.2, 105.4, 116.6, and 136.9 bp and most resembled the tRNA-PCR pattern obtained for *B. gaviniae*, but they lacked the *B. gaviniae* fragments of 88.2 and 239.5 bp. To our knowledge, no clinical cases with *Buttiauxella* strains have been described thus far.

#### CASE REPORT

In April 1998, a 31-year-old man was admitted to our hospital with a bifrontal contraction lesion and a D12-L4 fracture. Due to this trauma and neurological syndrome, he developed epileptic attacks, hyperflexion of the lower limbs, and a neurological urinary syndrome, wherefore a suprapubic catheter was placed. During a recent hospitalization the person suffered again from urinary retention and a urine sample was taken for further investigation. Microscopic examination of the urine sediment yielded 121 leukocytes per  $\mu$ l (normal count, less than 25 leukocytes per  $\mu$ l, as determined with UF100 [TOA Medical Electronics, Kobe, Japan]) and  $10^3$  bacteria per  $\mu$ l. Direct microscopy and culture indicated gram-negative rods at a concentration of  $10^6$  CFU/ml. All of these results are indicative of a urinary tract infection in a spinal cord patient.

The suprapubic catheter was removed and the patient was treated with ofloxacin (200 mg, twice a day) for 2 weeks. Therapy was successful, and no bacteria and no elevation in number of white blood cells were seen in later samples.

**Discussion.** Culture of the urine sample on tryptic soy agar with 5% sheep blood (Becton Dickinson, Erembodegem, Belgium) and on MacConkey agar (Becton Dickinson) yielded a lactose-negative gram-negative rod, designated LBV449. Iden-

tification with API 20E (Biomérieux, Marcy l'Etoile, France) resulted in code 3004113 or 1004113, due to a variable  $\beta$ -galactosidase reaction (*o*-nitrophenyl- $\beta$ -D-galactopyranosidase [ONPG] hydrolysis) that was negative in the latter case, which gave a good identification as *Escherichia vulneris* (96.5%) or a weak identification as *E. vulneris* (95%), *Buttiauxella agrestis* (85%), *Klebsiella pneumoniae* subsp. *ozaenae* (80%), or *Pantoea* sp. strain 4 (89%), respectively. Although lactose fermentation was scored negative, macro-testing showed that it was positive after 6 days of incubation. Lactose fermentation was found in this study to be slowly positive among *B. noackiae* strains but remained negative for the four *B. gaviniae* reference strains tested (S1/1 986, S1/1 987, S1/1 988, and S1/1 998).

The results of the biochemical characterization are presented in Table 1. The case report strain was negative for L-ornithine decarboxylase, melibiose, raffinose, and pyrrolidonyl aminopeptidase, which makes differentiation from *B. agrestis* possible. The case report strain could be differentiated from *B. noackiae* by a positive adonitol test and by the absence of phenylalanine deaminase. It was negative for ONPG hydrolysis and citrate in API 20E, but in macro-tests it was weakly or slowly positive for ONPG and citrate. No definite biochemical differentiation with *B. gaviniae* could be found when comparing these findings with previously published data (2–4, 8).

DNA-DNA hybridization was carried out as described previously (11). The isolation of DNA was done using the method of Marmur (6), with the additional steps of RNase A (Sigma, Steinheim, Germany) and RNase T1 (Sigma) treatment for 2 h at 37°C.

Our clinical isolate (LBV449) had DNA-DNA similarities of 42.3% to the type strain of *B. agrestis* (CDC 1176-81), 62% to

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TABLE 1. Strains investigated in this study and their biochemical characteristics

| Biochemical characteristic    | Test result by species, strain, and clinical or environmental source |                        |                                   |                                   |                                              |                      |                                                   |                           |                        |                                               |                              |                               |
|-------------------------------|----------------------------------------------------------------------|------------------------|-----------------------------------|-----------------------------------|----------------------------------------------|----------------------|---------------------------------------------------|---------------------------|------------------------|-----------------------------------------------|------------------------------|-------------------------------|
|                               | <i>B. agrestis</i>                                                   |                        |                                   |                                   | <i>B. gaviniiae</i>                          |                      |                                                   |                           | <i>B. noackiae</i>     |                                               |                              |                               |
|                               | WG 189, ENT1183, wound, right toe                                    | WG 190, ENT1194, human | ENB 141 S3/3, 203, snail, Germany | ENB 143 S3/3, 162, snail, Germany | ENB 142 CDC 17681, <sup>a</sup> soil, France | ENB148 ENT116, urine | (% Positive strains of 86), <sup>a</sup> mollusks | ENB153 ENT107, wound, leg | LBV 449, bladder urine | ENB 146 NSW 11, <sup>a</sup> snail, Australia | ENB 144 BR626, snail, Brazil | ENB 145 BR 489, snail, Brazil |
| Differentiating               |                                                                      |                        |                                   |                                   |                                              |                      |                                                   |                           |                        |                                               |                              |                               |
| Adonitol (macroscopic)        | —                                                                    | —                      | —                                 | —                                 | —                                            | —                    | 65                                                | +                         | +                      | —                                             | —                            | —                             |
| Arginine dihydrolase          | —                                                                    | —                      | —                                 | —                                 | —                                            | —                    | 100                                               | +                         | +                      | +                                             | +                            | +                             |
| Phenylalanine deaminase (APP) | +                                                                    | w <sup>b</sup>         | +                                 | +                                 | +                                            | +                    | 0                                                 | —                         | —                      | +                                             | +                            | +                             |
| Lactose                       | +                                                                    | +                      | +                                 | +                                 | +                                            | +                    | 1                                                 | Delayed +                 | Delayed +              | Delayed +                                     | Delayed +                    | Delayed +                     |
| Melibiose                     | +                                                                    | +                      | +                                 | +                                 | +                                            | +                    | 0                                                 | —                         | —                      | —                                             | —                            | —                             |
| Ornithine decarboxylase       | +                                                                    | +                      | +                                 | +                                 | +                                            | +                    | 0                                                 | —                         | —                      | —                                             | —                            | —                             |
| Pyrrolidonyl aminopeptidase   | +                                                                    | +                      | +                                 | +                                 | +                                            | +                    | ND <sup>c</sup>                                   | —                         | —                      | —                                             | —                            | —                             |
| Raffinose                     | —                                                                    | +                      | +                                 | +                                 | +                                            | +                    | 2                                                 | —                         | —                      | —                                             | —                            | —                             |
| Variable                      |                                                                      |                        |                                   |                                   |                                              |                      |                                                   |                           |                        |                                               |                              |                               |
| Citrate utilization (Simmons) | —                                                                    | +                      | +                                 | +                                 | +                                            | +                    | 36                                                | +                         | +                      | +                                             | +                            | +                             |
| Citrate (API, 30 and 37°C)    | —                                                                    | —                      | +                                 | +                                 | +                                            | +                    | 30                                                | —                         | —                      | —                                             | —                            | —                             |
| Indole                        | —                                                                    | —                      | —                                 | —                                 | —                                            | —                    | 25                                                | —                         | —                      | —                                             | —                            | —                             |
| Sorbitol                      | +                                                                    | —                      | —                                 | —                                 | —                                            | —                    |                                                   | —                         | —                      | —                                             | —                            | —                             |

<sup>a</sup> Data from Müller et al. (8).

<sup>b</sup> w<sup>+</sup>, weak positive.

<sup>c</sup> ND, not determined.

| Species            | Strain designation                | GenBank          | Sequence and <i>E. coli</i> position                          |     |      |      |      |      |
|--------------------|-----------------------------------|------------------|---------------------------------------------------------------|-----|------|------|------|------|
|                    |                                   | Accession Number | 980                                                           | 990 | 1000 | 1010 | 1020 | 1030 |
| <i>B. agrestis</i> | DSM 4586                          | AJ233400         | TACTCTTGACATCCAGAGAATTCGCTAGAGATAGCTTAGTGCCTTCGGGAACCTCTGAGAC |     |      |      |      |      |
| <i>B. agrestis</i> | ATCC 33320 <sup>T</sup> (ENB 142) | <b>AJ293685</b>  | *****SR*****C*ST*****AS*****YS*****                           |     |      |      |      |      |
| <i>B. agrestis</i> | ENB 141                           | <b>AJ293684</b>  | *****CG*****Y*GY*****GC*****CG*****                           |     |      |      |      |      |
| <i>B. agrestis</i> | ENB 143                           | <b>AJ293686</b>  | *****CR*****Y*GY*****GC*****YG*****                           |     |      |      |      |      |
| <i>B. gaviniae</i> | DSM 9393 <sup>T</sup>             | AJ233403         | *****CA*****C*GC*****GC*****TG*****                           |     |      |      |      |      |
| <i>B. gaviniae</i> | (this study, LBV449)              | <b>AJ293683</b>  | *****SA*****C*ST*****RS*****YS*****                           |     |      |      |      |      |
| <i>B. noackiae</i> | DSM 9401 <sup>T</sup>             | AJ233405         | *****CG*****C*GC*****GC*****CG*****                           |     |      |      |      |      |
| <i>B. noackiae</i> | ATCC 51607 <sup>T</sup> (ENB 146) | <b>AJ293689</b>  | *****CG*****Y*GC*****GC*****CG*****                           |     |      |      |      |      |
| <i>B. noackiae</i> | ENB 144                           | <b>AJ293687</b>  | *****CR*****Y*GC*****GC*****CG*****                           |     |      |      |      |      |
| <i>B. noackiae</i> | ENB 145                           | <b>AJ293688</b>  | *****SR*****Y*SC*****GS*****YS*****                           |     |      |      |      |      |

FIG. 1. 16S rRNA gene sequence alignments for *B. agrestis*, *B. gaviniae*, *B. noackiae*, and the *B. gaviniae* clinical isolate. Sequences with accession numbers shown in bold are those determined in this study. International Union of Microbiological Societies (IUMS) codes in the sequences: R = A or G; S = G or C; Y = C or T.

the type strain of *B. noackiae* (NSW 11), and 77.3% to a strain (ENT107) previously isolated from a leg wound which had been at that time identified as belonging to enteric group 59. The DNA-DNA similarity of LBV449 to the type strain of *B. gaviniae* (ATCC 51604) was 74%, and similarities to the type strains of all further *Buttiauxella* species (8) were in the range of 43 to 58%.

Cellular fatty acids were determined as described previously (5) and were of the straight-chain type, both saturated and unsaturated, with 16:0 and 16:19*cis* as major components. There was no significant difference between the cellular fatty acid profile of the various *Buttiauxella* species tested (4), although the data indicated that the highest similarity was between the clinical isolate and *B. noackiae*.

Genotypic identification was attempted by amplification of the 16S rRNA gene and sequencing (10). The 16S rRNA gene was amplified using the primers 5' AGT TTG ATC CTG GCT CAG 3' and 5' TAC CTT GTT ACG ACT TCG TCC CA 3'. Comparison of the sequence with all bacterial sequences available from the GenBank database using the Blast 2.0 program (National Center for Biotechnology Information, Bethesda, Md.) revealed similarities of between 99.2 and 99.8% with the different species of the *Buttiauxella* genus. However, alignment of the sequence obtained in this study for the clinical isolate and the GenBank sequences of *B. agrestis*, *B. gaviniae*, and *B. noackiae* (AJ233400, AJ233403, and AJ233405, respectively [9]) showed that there was sequence polymorphism in our strain in the only region (*Escherichia coli* position 980 to 1020) where these three species differ according to the GenBank entries. The ambiguities observed contained the bases that, according to published GenBank sequences, differentiate between the three species (Fig. 1). The purity of our strain was checked by reculturing, reisolation, and resequencing, with the same result. To study this phenomenon in more detail, six reference strains (three *B. agrestis* and three *B. noackiae*) were sequenced (Fig. 1). Again, the regions where the described differences are to be found were polymorphic, and the 16S ribosomal DNA sequences did not enable differentiation between the two species. Polymorphism in 16S rRNA genes is expected to be caused by microheterogeneities due to nucleo-

tide differences between the 16S rRNA genes present in multiple copies in the bacterial genome. The overall similarity of the published sequences of the *Buttiauxella* species is above 99% (9), but our data indicate that even several of the described differences are not really present. The difference between our results and those of Spröer et al. (9) remains unexplained, since those authors also applied direct sequencing and did not start from cloned 16S rRNA genes, which otherwise could have explained how they overlooked the ambiguities present.

tRNA-PCR (1, 7) enabled clear differentiation from *B. agrestis* and *B. noackiae* and from all clinically important gram-negative species (unpublished data, available upon request). The patterns obtained for the two clinical isolates (tRNA intergenic spacer lengths of 102.4, 105.4, 116.5, and 136.8 bp) resembled best those of *B. gaviniae*, with some distinct differences (Table 2).

Using the Kirby-Bauer disk diffusion method, the strain was found to be susceptible to ampicillin, cotrimoxazole, cefuroxime, gentamicin, furadantin, temocillin, and quinolones.

The clinical strain isolated from a urine sample from a spinal

TABLE 2. tRNA intergenic spacer lengths observed for *B. agrestis*, *B. gaviniae*, the two clinical *B. gaviniae* isolates of this study, and *B. noackiae*

| Avg length of tRNA spacer (SD) in bp | Presence of spacer in:              |                        |                                   |                        |
|--------------------------------------|-------------------------------------|------------------------|-----------------------------------|------------------------|
|                                      | <i>B. agrestis</i> (5) <sup>a</sup> | <i>B. gaviniae</i> (4) | <i>B. gaviniae</i> (clinical) (2) | <i>B. noackiae</i> (3) |
| 88.2 (0.01)                          | —                                   | +                      | —                                 | —                      |
| 102.2 (0.26)                         | —                                   | +                      | +                                 | —                      |
| 103.7 (0.11)                         | +                                   | —                      | —                                 | —                      |
| 105.4 (0.22)                         | (+) <sup>b</sup>                    | —                      | +                                 | +                      |
| 116.6 (0.32)                         | —                                   | +                      | +                                 | —                      |
| 136.9 (0.44)                         | +                                   | +                      | +                                 | —                      |
| 138.1 (0.07)                         | —                                   | —                      | —                                 | +                      |
| 225.5 (0.16)                         | —                                   | —                      | —                                 | +                      |
| 239.5 (0.06)                         | —                                   | +                      | —                                 | —                      |

<sup>a</sup> Number of strains tested.

<sup>b</sup> (+), present in most isolates.

cord patient with urinary bladder pathology had clinical significance and responded well to treatment. To our knowledge, only one clinical isolate (CDC 1650-80) belonging to *B. noackiae* has been described thus far (8). *B. gaviniae* has not been reported from clinical specimens until now, and all but one of the *B. gaviniae* isolates studied by Müller et al. (8) were isolated from snails.

In summary, 16S rRNA gene sequencing was indecisive because of the high degree of similarity and the presence of polymorphism in the described regions allowing differentiation. Also, fatty acid analysis was of little help. The two clinical isolates were positive for adonitol fermentation and L-arginine dihydrolase and negative for L-ornithine decarboxylase and melibiose and, thus, were biochemically indistinguishable from *B. gaviniae*. The tRNA-PCR patterns of these clinical isolates most resembled those of *B. gaviniae* and could be used to identify them as such.

**Nucleotide sequence accession number.** The DNA sequence of the clinical isolate obtained in this study was deposited in GenBank under accession number AJ293683.

#### REFERENCES

1. Baele, M., P. Baele, M. Vanechoutte, V. Storms, P. Butaye, L. A. Devriese, G. Verschraegen, M. Gillis, and F. Haesebrouck. 2000. Application of tRNA intergenic spacer PCR for identification of *Enterococcus* species. *J. Clin. Microbiol.* **38**:4201–4207.
2. Ferragut, C., D. Izard, F. Gavini, B. Lefebvre, and H. Leclerc. 1981. *Buttiauxella*, a new genus of the family *Enterobacteriaceae*. *Zentbl. Bakteriolog. Mikrobiol. Hyg. Abt. I Orig. C* **2**:33–44.
3. Gavini, F., D. Izard, C. Ferragut, J. J. Farmer, and H. Leclerc. 1983. Separation of *Kluyvera* and *Buttiauxella* by biochemical and nucleic acid methods. *Int. J. Syst. Bacteriol.* **33**:880–882.
4. Kämpfer, P., S. Meyer, and H. E. Müller. 1997. Characterization of *Buttiauxella* and *Kluyvera* species by analysis of whole cell fatty acid patterns. *Syst. Appl. Microbiol.* **20**:566–571.
5. Kämpfer, P., and R. M. Kroppenstedt. 1996. Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can. J. Microbiol.* **42**:989–1005.
6. Marmur, J. 1961. A procedure for the isolation of DNA from microorganisms. *J. Mol. Biol.* **3**:208–218.
7. McClelland, M., C. Petersen, and J. Welsh. 1992. Length polymorphisms in tRNA intergenic spacers detected by using the polymerase chain reaction can distinguish streptococcal strains and species. *J. Clin. Microbiol.* **30**:1499–1504.
8. Müller, H. E., D. J. Brenner, G. R. Fanning, P. A. D. Grimont, and P. Kämpfer. 1996. Emended description of *Buttiauxella agrestis* with recognition of six new species of *Buttiauxella* and two new species of *Kluyvera*: *Buttiauxella ferragutiae* sp. nov., *Buttiauxella gaviniae* sp. nov., *Buttiauxella brennerae* sp. nov., *Buttiauxella izardii* sp. nov., *Buttiauxella noackiae* sp. nov., *Buttiauxella warmboldiae* sp. nov., *Kluyvera cochlea* sp. nov. and *Kluyvera georgiana* sp. nov. *Int. J. Syst. Bacteriol.* **46**:50–63.
9. Spröer, C., U. Mendrock, J. Swiderski, E. Lang, and E. Stackebrandt. 1999. The phylogenetic position of *Serratia*, *Buttiauxella* and some other genera of the family *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* **49**:1433–1438.
10. Vanechoutte, M., G. Claeys, S. Steyaert, T. De Baere, R. Peleman, and G. Verschraegen. 2000. *Moraxella canis* isolation from an ulcerated metastatic lymph node. *J. Clin. Microbiol.* **38**:3870–3871.
11. Ziemke, F., M. G. Hofle, J. Lalucat, and R. Rossello-Mora. 1998. Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int. J. Syst. Bacteriol.* **48**:179–186.