Identification of Corynebacterium glucuronolyticum Strains from the Urogenital Tract of Humans and Pigs

LUC A. DEVRIESE, PHILIPPE RIEGEL, JOZEF HOMMEZ, MARIO VANEECHOUTTE, THIERRY DE BAERE, AND FREDDY HAEBEBROUCK

Faculty of Veterinary Medicine, University of Ghent, Merelbeke, Regional Veterinary Laboratory, Torhout, and Department of Chemistry, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium, and Laboratoire de Bactériologie, Faculté de Médecine, Hôpitaux Universitaires, Strasbourg, France.

Received 7 July 2000/Returned for modification 28 August 2000/Accepted 25 September 2000

Bacterial strains isolated from the genital tracts of humans (predominantly males), semen of boars, and uterine and vaginal secretions of sows were identified as Corynebacterium glucuronolyticum and were compared with the type strains of the recently proposed species Corynebacterium glucuronolyticum and Corynebacterium seminale. The two type strains as well as the clinical strains were shown by DNA-DNA hybridization and sequencing of the 16S rRNA gene to be related at the species level. All strains were classified as C. glucuronolyticum, because this name has nomenclatural priority over C. seminale.

During routine diagnostic investigations of clinical samples from pigs and examinations of boar semen samples, coryneform bacteria were detected that appeared to be biochemically very similar to the descriptions given of Corynebacterium glucuronolyticum (3) and Corynebacterium seminale (7).

During recent years, several new Corynebacterium species have been described from humans and animals. Species associated with the urogenital tract are remarkably well represented among the corynebacteria. Strains from prostatitis in humans were compared 16S rRNA gene sequencing and phylogenetic analysis and found to represent a distinct group within the genus Corynebacterium (10). This group was believed to comprise two named species: C. seminale and C. glucuronolyticum. However, recent reviews of the clinical significance of nomenclature and species descriptions of coryneform bacteria have indicated that C. glucuronolyticum is synonymous (4) or probably synonymous (6) to C. seminale, whereas in the study by Tanner and coworkers (10), the 16S ribosomal-DNA (rDNA) sequence of C. seminale differed up to 4% from that of C. glucuronolyticum. Finally, some of the strains on which the description of C. seminale was based do not hydrolyze esculin (7), while C. glucuronolyticum has been reported to be one of the very few human pathogenic Corynebacterium species that is able to hydrolyze this substrate (3).

The present investigation was undertaken in order to attain a definitive species identification of our strains. As this was not possible without clarification of the current nomenclatural and taxonomical situation, the porcine strains were compared genetically and phenotypically with human strains and with the type strains of C. glucuronolyticum and C. seminale.

All strains investigated were isolated from human or porcine urogenital specimens (Table 1). The field strains were isolated in France from humans and in Belgium from pigs. The porcine isolates were obtained from sows with abnormal vaginal discharge and from apparently normal semen samples from boars, all from different farms. The human isolates were obtained from semen (n = 6), the male urethra (n = 2), and the vagina (n = 1). Strains were grown on Columbia agar base (Oxoid, Basingstoke, United Kingdom) with 5% sheep blood in air supplemented with 5% CO₂. Growth characteristics and biochemical activities were studied as described earlier (5).

DNA was extracted from the two type strains (Culture Collection University Göteborg [CCUG] 33055T [C. glucuronolyticum] and Collection Institut Pasteur [CIP] 104297T [C. seminale]), four human strains, and one porcine strain (Table 1). Hybridization between labeled DNA and fragmented DNA preparations was carried out at 60°C for 16 h in 0.42 M NaCl by the S1 nuclease-trichloroacetic acid method (8).

16S rRNA gene sequences were determined as described earlier (11) for seven strains (Table 1). Transfer DNA (tDNA)-PCR was carried out as described previously (11).

All strains investigated showed the morphological and growth characteristics given in the species description of C. glucuronolyticum (3). They were gram-positive coryneform rods that produced white-yellowish circular convex colonies. No growth was observed at 25°C, poor growth was observed at 30°C, and less profuse growth occurred at 42°C than at 37°C. Supplementation with CO₂ enhanced growth. The strains were not hemolytic, but they produced a CAMP effect with staphylococcal β-hemolysin. They were catalase-, β-glucuronidase, pyrazinamidase, and leucine arylamidase positive and produced acid from glucose and sucrose. Other reactions that were positive with all strains included amylase, L-phenylalanine-7-amino-4-methylcoumarin (L-phenylalanine-AMC), L-tryptophan-AMC, L-arginine-AMC, and 4-methylumbelliferyl (MU)-β-D-glucuronide, with the latter four tests being part of the BBL CRYSTAL gram-positive identification system. None of the strains produced alkaline phosphatase in the API CORYNE and API 20 STREP galleries. Hydrolysis of esculin, p-nitrophosphoryl-β-D-glucoside, and 4-MU-β-D-glucoside and acid production from methyl-α and β-glucoside, arbutin, and salicin (Table 2) were uniformly negative for the porcine strains and for human clinical strains Institut Bactériologie Strasbourg (IBS) B5856, IBS B46176, and IBS B49925 as well as for strain CIP 104297T, the type strain of C. seminale (biochemical group I), but they were positive for the C. glucuronolyticum type strain CCUG 33055T and for the four remaining human strains (biochemical group II). Other reactions were variable in these two groups. Two other clinical strains (IBS B22035 and IBS Dev, placed here in biochemical group III)
showed reactions differing from those of groups I and II (Table 2).

The labeled DNA of C. glucuronolyticum type strain CCUG 33055T exhibited 78% similarity with the C. seminale type strain CIP 104297T, 88% with the pig strain 3992.2, and 74%, 81%, 84%, and 100% similarity with human strains IBS B46171, IBS B49925, IBS STEC, and IBS Mar, respectively. The seven sequences determined during this study were more than 98.2% similar to each other and to a previously published C. seminale sequence (7). Only 98.2% similarity was observed with C. glucuronolyticum strain DSM 44120 (GenBank accession no. X86688). tDNA-PCR was carried out for the 11 human strains and 6 pig strains. All human strains had a very similar tDNA-PCR fingerprint, with three peaks in common having an average length of respectively, 57.4 bp (standard deviation, ±0.07 bp), 60.2 bp (±0.05 bp), and 332.6 bp (±0.12 bp). The six pig strains had tDNA spacer fragments with average lengths of 60.1 bp (±0.09 bp), 70.2 bp (±0.02 bp), 98.9 bp (±0.05 bp), and 318.5 bp (±0.14 bp) in common. These tDNA spacer profiles were not observed for any of 25 other corynebacterial species tested or for any of 49 other gram-positive species.

The genotypic characterizations of C. seminale and C. glucuronolyticum type strains and clinical human and porcine isolates carried out in this study clearly indicate that the strains described as C. seminale (7) and those described as C. glucuronolyticum (3) belong to the same species. Both names were proposed almost simultaneously, but the name C. glucuronolyticum was published first and has nomenclatural priority (1, 2). The biochemical activity of the different strains investigated did not differ from key reactions described for this species (3), except for alkaline phosphatase, which was found to be negative in the present investigation, confirming the description given in the paper describing C. seminale (7). Some possible host-species-associated characteristics were evident. The porcine isolates reacted biochemically homogeneously, and certain human strains showed exactly the same reaction profiles as the pig strains, while others differed more or less. tDNA-PCR enabled unambiguous differentiation between the studied human and porcine strains.

Apparently, the habitat of this species is not limited to humans. The species is also associated with at least one other mammalian host, where it occurs in the same body site as in humans, namely the urogenital tract. These observations confirm and extend the report of Takahashi et al. (9) on the similarity of the nucleotide sequence of a Japanese strain, isolated from the cervical canal of a sow, with that of C. seminale. The clinical relevance of C. glucuronolyticum for pigs remains unknown, and also the clinical significance of the human isolates was difficult to establish. Strains IBS B51078, IBS V3467, IBS B46176, IBS B49925, IBS STEC, and IBS Dev were isolated from semen of men suffering from infertility, with a bacterial count of more than 10,000 CFU/ml; strains IBS B22035 and IBS B5856 were from men with urethritis; and strain IBS Mar was from a woman with vaginosis. C. glucuronolyticum isolates were numerous and predominant in all cases.
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ERRATA

Rapid and Specific Genotyping System for Hepatitis B Virus Corresponding to Six Major Genotypes by PCR Using Type-Specific Primers
HIDEO NAITO, SHIGEKI HAYASHI, AND KENJI ABE
Department of Pathology, National Institute of Infectious Diseases, and Division of Gastroenterology, International Medical Center of Japan, Tokyo, Japan

Volume 39, no. 1, p. 362–364, 2001. Page 363, Table 1: The sequence for primer BF1 should read “5’-GYT ACG GTC CAG GGT TCA CA-3’.”

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LUC A. DEVRIESE, PHILIPPE RIEGEL, JOZEF HOMMEZ, MARIO VANEECHOUTTE, THIERRY DE BAERE, AND FREDDY HAESEBROUCK
Faculty of Veterinary Medicine, University of Ghent, Merelbeke, Regional Veterinary Laboratory, Torhout, and Department of Chemistry, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium, and Laboratoire de Bactériologie, Faculté de Médecine, Hôpitaux Universitaires, Strasbourg, France

Page 4657, column 2, line 4 from bottom: “CCUG 33055 T” should read “CCUG 35055 T.”
Page 4658, column 1, lines 3 and 4: “CCUG 33055 T” should read “CCUG 35055 T.”
Page 4658, Table 1, row 1: “CCUG 33055 T” should read “CCUG 35055 T.”

Species-Specific Identification of Human Adenoviruses by a Multiplex PCR Assay
WANHONG XU, MIKE C. MCDONOUGH, AND DEAN D. ERDMAN
Respiratory and Enteric Viruses Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Volume 38, no. 11, p. 4114–4120, 2000. Page 4115, column 1, line 14 from the bottom: “0.2 mM each primer” should read “0.2 μM each primer.”