Phenotypic Differentiation between *Clostridium hastiforme* and *Clostridium subterminale* by Headspace Gas Chromatography

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Assessment of methane and methanethiol in the headspace gas from cultures of various strains labeled *Clostridium hastiforme* or *Clostridium subterminale* allowed differentiation between these two species. *C. hastiforme* strains produced significantly more methane and less methanethiol than *C. subterminale* strains. We confirm that strain DSM 1786 (= ATCC 25772) does not belong to either of these species.

Bacteria of the genus *Clostridium* form a very heterogeneous group (3), and their identification requires both conventional phenotypic tests and analysis of volatile fatty acids by gas chromatography (4). As in some instances these procedures are not sufficient to assign a strain to a nomen-species and to differentiate some taxa, additional criteria are needed (2). The simultaneous analysis of hydrocarbons and volatile organosulfur compounds by headspace gas chromatography is a convenient approach for the study of clostridia (5). In this paper, we report that this headspace gas chromatography method can be helpful for distinguishing between *Clostridium hastiforme* and *Clostridium subterminale*.

We examined 10 strains in this study, including the type strains of *C. hastiforme* and *C. subterminale*. The sources of

compounds by gas chromatography. Separation was achieved on columns packed either with *n*-octane–Porasil C or with a 0.5-nm (aperture size) molecular sieve for the quantification of methane. Details of the method which we used have been described previously (5, 6).

The headspace gas chromatography analysis of hydrocarbons and volatile organosulfur compounds was applied to four strains labeled *C. hastiforme*, to five strains labeled *C. subterminale*, and to an unnamed strain, *Clostridium* sp. strain MPP 20 (= DSM 1786 = ATCC 25772 = VPI 2461). Our results indicate that these strains can be classified into three groups, according to the detection of methane and methanethiol (Table 2). *C. hastiforme* strains all produced more than 40 nmol of methane per tube and a methanethiol peak area less than 200 cm². *C. subterminale* strains all

TABLE 1. List of strains of *Clostridium* species and their sources

Strain ^a	Species	Received from ^b :	Source
MPP 22 ^{Tc}	<i>C. hastiforme</i>	ATCC (ATCC 33268 ^T)	Abdominal abscess (California)
MPP 113	<i>C. hastiforme</i>	Cato (VPI 10426)	Bovine brain (California)
MPP 114	<i>C. hastiforme</i>	Cato (VPI 11497A)	Blood (Minnesota)
MPP 115	<i>C. hastiforme</i>	Cato (VPI 12486A)	Leg wound (Texas)
MPP 21 ^T	<i>C. subterminale</i>	ATCC (ATCC 25774 ^T)	Unknown
MPP 116	<i>C. subterminale</i>	Cato (VPI 8675A)	Wound (Maryland)
MPP 117	<i>C. subterminale</i>	Cato (VPI 8677)	Foot wound (Texas)
MPP 118	<i>C. subterminale</i>	Cato (VPI 8716)	Perineum wound (Georgia)
MPP 137	<i>C. subterminale</i>	Sebald (AIP 1992)	Unknown
MPP 20	<i>Clostridium</i> sp.	DSM (DSM 1786)	Postappendectomy peritonitis (Lille, France)

^a MPP, Microbiologie Pharmacie Paris, Paris, France.

^b ATCC, American Type Culture Collection, Rockville, Md.; Cato, E. P. Cato, Virginia Polytechnic Institute and State University (VPI), Blacksburg; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; Sebald, M. Sebald, Unité des Anaérobies, Institut Pasteur (AIP), Paris, France.

^c T = type strain.

these strains are listed in Table 1. All of the strains were cultured at 37°C under reduced pressure in 5 ml of TTY medium containing (per 1,000 ml of distilled water) 15 g of Trypcase (BioMérieux, Lyon, France), 5 g of yeast extract (BioMérieux), 2.5 g of sodium chloride (E. Merck AG, Darmstadt, Federal Republic of Germany), and 0.5 g of sodium thioglycolate (Merck). Triplicate preparations for each strain and duplicate preparations for uninoculated medium were processed in the same way.

Reduced-pressure headspaces from 168-h cultures were analyzed for light hydrocarbons and volatile organosulfur

produced less than 20 nmol of methane per tube and a methanethiol peak area greater than 8,000 cm². No overlapping of the results was observed. The third group corresponded to one strain, *Clostridium* sp. strain MPP 20, which cannot be included in the other groups.

C. hastiforme and *C. subterminale* share many phenotypic properties (2). These nonglucidolytic clostridia show moderate proteolysis, with *C. hastiforme* being somewhat less proteolytic than *C. subterminale* (8). Protein electrophoresis is a useful tool for distinguishing the two species (2). As a consequence, strain ATCC 25772 (= MPP 20), formerly the type strain of *C. hastiforme* (7), was placed in the unnamed clostridium group, while strain ATCC 33268 (= VPI 12193) was designated as the new type strain of *C. hastiforme* (2).

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TABLE 2. Methane and methanethiol detected in headspace of TTY medium cultures of four *C. hastiforme* strains, five *C. subterminale* strains, and one *Clostridium* sp. strain

Strain or prepn	Amt of methane (nmol/tube) ^{a,b}	Methanethiol peak area (cm ²) ^{a,c}
<i>C. hastiforme</i> MPP 22 ^T	408 (13)	109 (3)
<i>C. hastiforme</i> MPP 113	214 (7)	172 (1.5)
<i>C. hastiforme</i> MPP 114	260 (5)	172 (0.6)
<i>C. hastiforme</i> MPP 115	43 (0.4)	172 (10)
<i>C. subterminale</i> MPP 21 ^T	8 (0.6)	8,700 (400)
<i>C. subterminale</i> MPP 116	7 (0.3)	17,500 (200)
<i>C. subterminale</i> MPP 117	6 (0.1)	15,800 (160)
<i>C. subterminale</i> MPP 118	15 (0.5)	15,800 (1100)
<i>C. subterminale</i> MPP 137	12 (0.3)	17,200 (200)
<i>Clostridium</i> sp. strain MPP 20	64 (0.3)	14,900 (850)
Uninoculated medium	0.50 (0.3)	22 (2)

^a Means were calculated from triplicate experiments for each culture and from duplicate experiments for uninoculated medium. Values in parentheses are standard deviations.

^b Results can be expressed in nanomoles per tube because methane is distributed only in the gas phase (5, 6).

^c Results are expressed arbitrarily as peak areas in square centimeters (triangulation), because methanethiol is distributed in liquid and in gas phases and the amount detected in the headspace reflects the amount in the medium, but quantitative evaluation was hitherto impracticable.

Spore location, production of hydrogen (2), and sodium citrate utilization (1) can be other discriminating factors. Results obtained from headspace gas chromatography patterns confirmed that strains labeled *C. hastiforme* form one group, strains labeled *C. subterminale* form a second group, and strain ATCC 25772 does not belong to either species. Recent work (9) has individualized *Clostridium argentinense* and has divided the other strains initially identified as *C. hastiforme* or *C. subterminale* into six deoxyribonucleic acid relatedness groups. Unfortunately, our study and the study of Suen et al. (9) had in common only the type strains of *C. hastiforme* and *C. subterminale* and *Clostridium* sp. strain ATCC 25772 (= AL 1449 = MPP 20). Our results and those of Suen et al. are in perfect agreement on this point; *Clostridium* sp. strain ATCC 25772 is neither a *C. hastiforme* strain nor a *C. subterminale* strain.

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