JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1986, p. 1098–1099
0095-1137/86/121098-02$02.00/0

Vol. 24, No. 6

NOTES

Rapid Differentiation of Streptococci Isolated from Cows with Mastitis

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Received 31 March 1986/Accepted 19 August 1986

The demonstration of some species-specific streptococcal enzymes with 4-methylumbelliferyl-conjugated β-D-glucuronide, *N*-acetyl-β-D-glucosaminide, and β-D-mannoside and agglutination with the lectin from *Dolichos biflorus* allowed rapid tentative identification of streptococci isolated from cows with mastitis.

Several diagnostic methods are available for the differentiation of streptococci isolated from cows with mastitis. They include biochemical and serological procedures (2, 5), as well as CAMP reactions (3). The results of most of these tests can be obtained after an incubation of 18 to 24 h. Recently, Slifkin and Gil (7) described a more rapid identification method of streptococci from human infections with 4-methylumbelliferyl-conjugated substrates. These substrates served for the detection of species-specific streptococcal enzymes. The respective tests required an incubation period of only 20 min. In addition, the lectin of *Dolichos biflorus* was used to agglutinate streptococci of serological group C. A similar enzyme test proved useful for the differentiation of B streptococci isolated from infected humans and cows with mastitis (4).

In the present study, the 4-methylumbelliferyl-conjugated substrates β-D-glucuronide, *N*-acetyl-β-D-glucosaminide, and β-D-mannoside and the lectin of *D. biflorus* served for tentative identification of streptococci from cows with mastitis.

A total of 92 streptococcal cultures of bovine mastitis isolates were studied. The streptococci were identified (3) and serogrouped. For serogrouping, nitrous acid extracts were prepared from the streptococci (1) and tested with group-specific antisera (Wellcome Research Laboratories, Burgwedel, Federal Republic of Germany) by agar gel diffusion. The 92 streptococcal cultures included 20 of serological group B (*S. agalactiae*), 26 of group C (*S. dysgalactiae*), 25 of group D (6 cultures of *S. durans*, 4 of *S. faecium*, 13 of *S. faecalis*, and 2 of *S. bovis*), 5 of group G, and 16 of *S. uberis*. The 4-methylumbelliferyl-conjugated substrates β-D-glucuronide and *N*-acetyl-β-D-glucosaminide were prepared as described by Slifkin and Gil (7). In addition, 4-methylumbelliferyl-β-D-mannoside (Sigma Chemical Co. Deisenhofen, Federal Republic of Germany) at a final concentration of 15 μmol/liter was dissolved in 0.2 ml of dimethylsulfoxide and supplemented to a volume of 10 ml with 0.2 mol of acetate buffer per liter (pH 5.2). For enzyme assays, 20 colonies of each streptococcal culture were suspended in 20 μl of the respective 4-methylumbelliferyl-conjugated substrate solutions in test tubes (5 by 45 mm). After an incubation of 1 to 2 h at 37°C and the addition of 20 μl of 0.1 N NaOH, a positive reaction was indicated by the development of a distinct fluorescence at 365 nm.

For the lectin agglutination test, 1 mg of lectin from *D. biflorus* (Sigma) was suspended in 1 ml of phosphate-buffered saline (0.15 mol/liter; pH 7.5) and the suspension was diluted 1:8 in the same buffer. Of this lectin dilution, 20

TABLE 1. Differentiation of streptococcal cultures of bovine mastitis isolates by species-specific enzyme activities

Streptococcal culture and serogroup	No. tested	Reaction with 4-methylumbelliferyl-conjugated substrate or lectin			
		β-D-Glucuronidase	*N*-Acetyl-β-D-glucosaminidase	β-D-Mannosidase	Agglutination with *D. biflorus* lectin
S. agalactiae, B	20	+	−	−	−
S. dysgalactiae, C	26	+	+[a]	−	+
D	25	−	+[b]	+	−
G	5	+	+	−	−
S. uberis	16	+	+[c]	+	−

[a] Total of 20 positive and 6 negative reactions.
[b] Total of 23 positive and 2 (*S. bovis*) negative reactions.
[c] Total of eight positive and eight negative reactions.

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μl was added to 20 μl of streptococcal suspension containing approximately 20 colonies in phosphate-buffered saline on a microscope slide. After the slide was rotated for 1 min, a distinct agglutination of the bacteria indicated a positive reaction.

The reaction of species-specific enzymes of the streptococci with the 4-methylumbelliferyl-conjugated β-D-glucuronide, *N*-acetyl-β-D-glucosaminide, and β-D-mannoside and the agglutination of group C streptococci with the lectin of *D. biflorus* allowed differentiation of streptococci isolated from cows with mastitis (Table 1). *S. agalactiae* (group B) and *S. dysgalactiae* (group C) split 4-methylumbelliferyl-β-D-glucuronide. However, only *S. dysgalactiae* agglutinated with the lectin of *D. biflorus*. Furthermore, α-hemolytic *S. dysgalactiae* could be differentiated from the other, distinctly β-hemolytic streptococcal species of group C. Streptococci of group D did not produce β-D-glucuronidase, in contrast to *S. uberis*, which contained no group-specific antigen and therefore could not be identified by serogrouping. Streptococci of group D and *S. uberis* formed β-D-mannosidase. None of the other streptococcal species tested produced this enzyme. Streptococci of serological group G formed β-D-glucuronidase and *N*-acetyl-β-D-glucosaminidase in accordance with the previous observations of Slifkin and Gil (7). The demonstration of species-specific enzyme activities of the streptococci with the 4-methylumbelliferyl-conjugated substrates in combination with the lectin agglutination of group C streptococci allowed differentiation within 1 to 2 h at 37°C. On the other hand, the API 20 Strep system described by Poutrel and Ryniewicz (6) required an incubation period of 4 to 24 h. Thus, 4-methylumbelliferyl-conjugated substrates in combination with a lectin from *D. biflorus* proved to be suitable for the rapid identification of streptococci from cows with mastitis.

This study was supported by Deutsche Forschungsgemeinschaft, Bonn, Federal Republic of Germany.

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