JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1990, p. 628–630
0095-1137/90/030628-03$02.00/0

Vol. 28, No. 3

Whole-Cell Protein Patterns of Nonhemolytic Group B, Type Ib, Streptococci Isolated from Humans, Mice, Cattle, Frogs, and Fish

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Received 11 October 1989/Accepted 6 December 1989

Whole-cell protein and physiological patterns of nonhemolytic group B, type Ib, streptococci isolated from humans, cattle, frogs, fish, and mice were compared. Isolates from humans, fish, and mice were identical. Only minor differences were seen in the isolates from human, bovine, and frog sources. Nonhemolytic group B streptococci from humans, fish, and mice and, to a lesser extent, from cattle and frogs share several characteristics, including a high similarity in proteins (on the basis of molecular weight); this suggests that they may have a common ancestry.

Group B streptococci are isolated primarily from cattle or humans. They have rarely been recovered from infections in camels (4), dogs (8), cats (3), fish (14), frogs (1), and hamsters (9), indicating that these bacteria have a broad host range. The bacteria are predominantly beta-hemolytic (97%), but occasional nonhemolytic strains have been isolated (5). Serologically identical nonhemolytic group B, type Ib, streptococci have been isolated from human (16), fish (16), frog, bovine, and mouse (13) sources. The isolation of apparently closely related bacteria causing disease in both cold-blooded aquatic organisms and warm-blooded hosts is unusual. To determine whether these isolates are genetically related, we compared their whole-cell protein profiles after separation by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Nonhemolytic group B, type Ib, streptococci isolated from humans (eight isolates), fish (five isolates), frogs (two isolates), and cattle (two isolates) and from an endemic outbreak of suppurative meningoencephalomyelitis in a nude mouse colony (13) (1 isolate) were recovered from our culture collection. The sources of isolation of the bacteria and their biochemical reactions are listed (Table 1). The bacteria were grown in 250 ml of Todd-Hewitt broth for 24 h at 35°C, pelleted at 16,000 × *g* for 20 min and frozen. After 24 h, the bacteria were thawed to room temperature and treated with a Tris-EDTA-lysozyme solution (50 mM Tris, 1 mM EDTA, 10 mg of lysozyme per ml [pH 6.6]) for 2 h at 37°C. The bacteria were then sonicated at 80 W in 15-s bursts for a total of 2 min with cooling. After low-speed centrifugation to remove bacterial debris, the supernatant fluid was pelleted at 100,000 × *g* for 1 h. The pellet was then suspended in distilled water, the protein concentration was determined by the method of Lowry et al. (11), and the concentration was adjusted to 2 mg/ml with distilled water.

The whole-cell proteins were separated in an 11% acrylamide gel by using standard SDS-polyacrylamide gel electrophoresis procedures (10). The samples were prepared by adding 25 μl of the whole-cell suspension to an equal volume of Tris–glycerol–2-mercaptoethanol–SDS buffer (0.5 M Tris, 0.20 M glycerol, 0.10 M 2-mercaptoethanol, 0.014 M SDS [pH 6.8]) containing 2 mg of the internal standards phosphorylase *b* (97,400 daltons [Da]; Sigma Chemical Co., St. Louis, Mo.) and soybean trypsin inhibitor (20,100 Da; Sigma) per ml. The samples were heated for 5 min at 100°C, and 25 μl was added to the wells. Electrophoresis was at 32.5 mA per gel at 10°C. After electrophoresis, the gels were fixed overnight with 50% methanol–10% acetic acid, stained for 2 h with 0.125% Coomassie brilliant blue R-250 (Bio-Rad

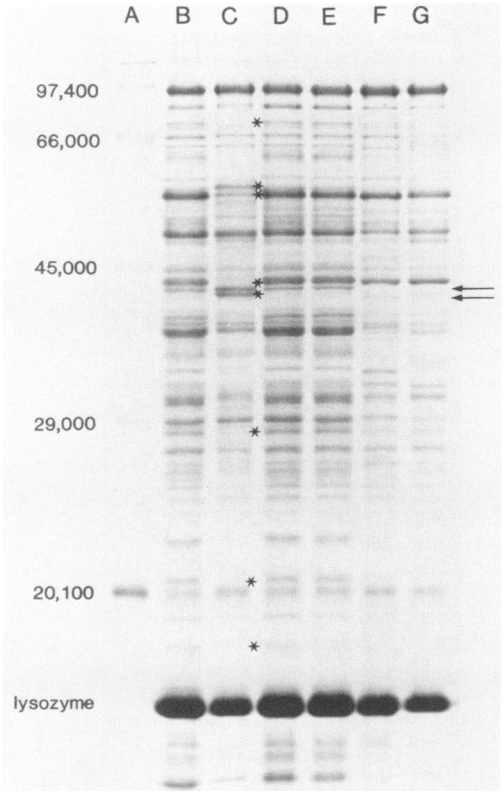

FIG. 1. SDS-polyacrylamide gels of the whole-cell proteins of nonhemolytic group B, type Ib, streptococci. Lane A, Molecular weight standards phosphorylase *b* (97,400 Da), bovine serum albumin (66,000 Da), ovalbumin (45,000 Da), carbonic anhydrase (29,000), and soybean trypsin inhibitor (20,400 Da); lane B, mouse isolate; lane C, bovine isolate (asterisks indicate proteins different from those of the human isolate); lane D, fish isolate; lane E, human isolate; lanes F and G, frog isolates (arrows indicate proteins different from those of human, mouse, and fish isolates).

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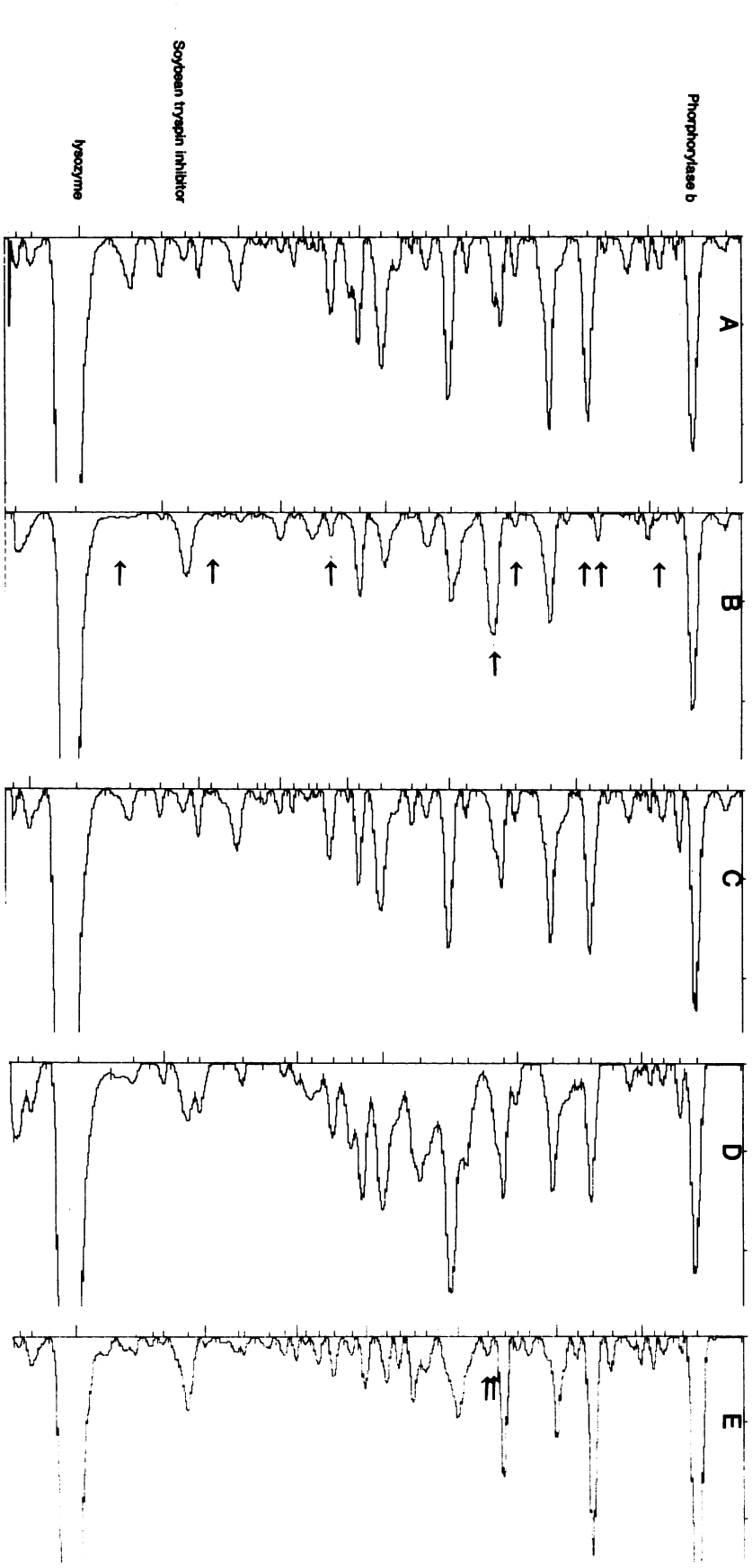

FIG. 2. Densitometer profiles. Scan A, Mouse isolate; scan B, bovine isolate (arrows indicate protein differences from the human isolate and correspond to asterisks in Fig. 1, lane C); scan C, fish isolate; scan D, human isolate. The two isolates from frogs had identical profiles, and the scan of Fig. 1, lane F, is shown in scan E (arrows indicate protein differences from human, mouse, and fish isolates).

TABLE 1. Biochemical characteristics of nonhemolytic group B, type Ib, streptococci[a]

Source and no. of isolates	Result for:					
	4% NaCl	Salicin	Trehalose	Litmus milk	CAMP[b]	Lactose
Mouse (1)	+	+	+	−	+	−
Cow (2)	+	+	+	A/C[c]	+	+
Fish (5)	+	+	+	−	+	−
Human (8)	+	+	+	−	+	−
Frog (2)	−	−	−	−	−	−

[a] The isolates hydrolyzed hippurate; were susceptible to bacitracin; were positive for the fermentation of sucrose; were negative for the fermentation of sorbitol, mannitol, inulin, raffinose, glycerol, arabinose, and sorbose; and were negative for esculin and bile esculin.
[b] Christie, Atkins, and Munch-Petersen reaction (2).
[c] Acid with a clot.

Laboratories, Richmond, Calif.) in the same solution, and destained with several changes of 5% methanol–7% acetic acid. After the gels were destained to a clear background, they were scanned with a soft laser densitometer (Biomed Instruments, Inc., Fullerton, Calif.), and the scans were normalized and compared by using a flexible superimposition program (Biomed Instruments, Inc.).

The SDS-polyacrylamide gel of representative isolates is shown in Fig. 1, and the densitometer scans of the proteins are shown in Fig. 2. The mouse, fish, and human isolates have identical protein patterns (Fig. 1, lanes B, D, and E; Fig. 2, scans A, C, and D). The bovine isolate was similar to the human isolate but differed both in proteins, primarily in the 30,000- to 50,000-Da range (Fig. 1, lane C; Fig. 2, scan B), and in fermentation of lactose (Table 1). Although there was significantly less whole-cell protein extracted from the bacteria isolated from the frogs (Fig. 1, lanes F and G), the scan (Fig. 2, scan E) of the protein patterns revealed only a few minor differences between the frog and the human, mouse, and fish isolates (Fig. 1, lane G; Fig. 2, scan E). The frog isolates differed from the other nonhemolytic group B isolates by the CAMP reaction (2) and by not growing in 4% NaCl. In addition, they did not ferment salicin or trehalose (Table 1). Both isolates from frogs had identical protein patterns (Fig. 1, lanes F and G).

Whole-cell protein patterns have been used by several investigators to determine the genetic similarities of groups of bacteria (6, 7). This technique is believed to be comparable to DNA-DNA hybridization methods (12). Wanger and Dunny used DNA-DNA hybridization to show that hemolytic group B streptococci with different serotypes, isolated from humans and bovine sources, are genetically related (15). Our report extends an earlier study on the physiological characteristics of several nonhemolytic group B, type Ib, streptococci that were isolated from humans and fish (16) and indicates that bacteria with the same protein profiles (Fig. 1 and 2) and similar biochemical utilization patterns (Table 1) can also be isolated from mice and frogs. The great similarity between the whole-cell protein profiles of the isolates from frogs, fish, humans, and mice suggests that they all share a common ancestor. The differences in the biochemical utilization by the frog isolates may indicate phenotypic adaptations to a host. Unlike the results indicating genetic similarity of hemolytic group B streptococci isolated from human and bovine sources described by Wanger and Dunny (15), our whole-cell protein pattern results indicate that the nonhemolytic bovine group B streptococci have a different or more distant ancestor or have undergone genetic changes. As indicated by the previous report (16), no evidence that these bacteria can be transmitted between the various animal species exists, although the possibility of transmission has not been determined.

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