Phage Heterogeneity of Coagulase-Negative Staphylococci Isolated in the United States and Sweden from Bovine Milk†

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A Swedish bovine and a Dutch human phage set for coagulase-negative staphylococci were used to phage type coagulase-negative staphylococci isolated from bovine milk from Minnesota dairy herds. A comparison was also made of the deoxyribonuclease activity of coagulase-negative staphylococci isolated from bovine milk in Sweden and in Minnesota. Of 133 Minnesota isolates, only one could be typed with the Swedish set and one by the Dutch set, whereas of 218 Swedish strains, 49 could be typed with the Swedish set and 7 by the Dutch set. A larger number of coagulase-negative isolates from Sweden were deoxyribonuclease positive (35%) than were the similar isolates from Minnesota (12%). These findings substantiate the marked heterogeneity of coagulase-negative staphylococci isolated from bovine udders. Results presented point to the usefulness of establishing regional phage sets for epidemiological investigations of coagulase-negative staphylococci in cattle. It is anticipated that at a later stage the regional phage sets will be coordinated internationally.

Materials and Methods

A total of 133 American and 218 Swedish coagulase-negative staphylococcus isolates from quarter samples of 141 and 180 cows, respectively, in 10 Minnesota and 5 Swedish herds were tested. Total somatic cell counts per milliliter of milk sample ranged between 41,000 and 5,000,000, as determined by the direct microscopic cell count method (11).

Phage typing. Phage typing was carried out by using the method described by Blair and Williams (4). The human phage set of Verhoef of the Netherlands (thesis, 1970) (Dutch phage set) and the bovine phage set of Holmberg (8) (Swedish phage set) were used for typing. The phages isolated from human coagulase-negative staphylococci (Verhoef, thesis) are: 48, 51, 71, 71A, 82, 82A, 108, 130, 130A, 157, 275, 275A, 275B, 448, 456, 459, 471, and 489. The phages isolated from bovine coagulase-negative staphylococci (9) are: 4, 10, 23, 39, 41, 49, 50, 156, 177, 243, and 266. Typing was performed at routine test dilution (RTD) and RTD ×100. RTD is defined as the highest test dilution of phage producing an almost confluent lysis.

Induction of phages. The induction of phages from coagulase-negative staphylococci isolated in Minnesota was carried out by the multiple-cross broth culture technique described by Rose and McDonald (12). Forty strains were divided into four groups. Four composite cultures were made by transferring a portion of representative colonies from each group to 5 ml of nutrient broth. After incubation at 37°C for 4 h on a reciprocal shaker, the composite cultures were centrifuged at 2,000 × g for 20 min at 5°C. One drop of each of the four supernatants was placed on the surface of each agar plate used for typing.

DNase test. The deoxyribonuclease (DNase) test was performed by using the method of Jeffries et al.

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RESULTS AND DISCUSSION

Other investigators have shown that coagulase-negative staphylococci, based on their biochemical properties, form a very heterogeneous group (1-3, 5, 6, 13, 14). Biochemical heterogeneity is not as marked among coagulase-positive strains. Coagulase-negative strains with differing biochemical properties may be isolated from the same quarter of a staphylococcus-infected bovine udder (Holmberg, unpublished data). This is seldom seen in S. aureus-infected udders. Differences in phage patterns of coagulase-negative and coagulase-positive strains have been observed. Thus, it was shown by Holmberg, using phage typing as a parameter, that the relationship between human and bovine coagulase-negative staphylococci was less than between human and bovine coagulase-positive staphylococci (8).

In this study we chose to compare coagulase-negative staphylococci isolated from dairy cattle in Minnesota and those isolated in Sweden with respect to their phage types and DNase activities. The Swedish and Dutch sets were used for typing. This investigation showed that differences do indeed exist among coagulase-negative strains isolated from bovine milk in widely separated geographical areas. For example, only one bovine strain of 133 Minnesota isolates could be phage typed with the Swedish (bovine) set and one by the Dutch (human) set. On the other hand, of 218 Swedish bovine coagulase-negative strains, 49 (23%) could be typed with the Swedish set and 7 (3%) with the Dutch set. The phage induction procedure of Rose and McDonald (12), in which Minnesota strains were employed, revealed lysis of 8 of the 133 Minnesota coagulase-negative isolates (6%).

A difference was also noted in the coagulase and DNase activities of the isolates from Sweden and Minnesota. Sixteen (12%) of the Minnesota coagulase-negative isolates produced DNase, and 16 (12%) were DNase intermediate. In contrast, 75 (35%) of the 218 coagulase-negative bovine isolates from Sweden produced DNase. More DNase-positive Swedish strains than Minnesota strains could be phage typed, 41 versus 13%, respectively.

Phage typing is recognized as a valid procedure for the characterization of coagulase-positive staphylococci of human origin (4). The same procedure, when properly standardized, should serve a similar purpose for coagulase-negative staphylococci of bovine origin. However, phage from certain geographic areas will not lyse coagulase-negative staphylococci from other areas, indicating heterogeneity. The phage types and DNase activity of coagulase-negative staphylococci of bovine origin isolated in Sweden are markedly different from those of the Minnesota isolates. This does not rule out the validity of phage typing as a means of characterizing coagulase-negative staphylococci. Rather, it lends support to the thesis of the heterogeneity of this particular group of microorganisms. Results of this investigation argue for the establishment of regional sets for epidemiological investigations of coagulase-negative staphylococci in cattle. It is anticipated that at a later stage the regional phage sets will be coordinated internationally.

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LITERATURE CITED


