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Elastolytic Activity among Staphylococci

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A total of 161 isolates of the genus *Staphylococcus* were evaluated for the ability to produce elastase. Elastase activity was detected only in *S. epidermidis* strains (sensu stricto), being absent in *S. aureus* isolates and other coagulase-negative staphylococci tested. The elastase elaborated by *S. epidermidis* isolates appeared to be an inducible enzyme whose synthesis was medium dependent.

Coagulase-negative staphylococci (CNS) are increasingly being recognized as important nosocomial pathogens commonly involved in neonatal bacteremia, endocarditis, urinary tract infections, and in complications arising from catheterization or hyperalimentation procedures (1, 12). Of the 11 CNS species known to colonize or infect humans (7), by far the most frequently documented agent is *S. epidermidis* (sensu stricto), an organism comprising up to 70% of all biochemically identified CNS. Although regarded as an emerging nosocomial pathogen, little information is available regarding what virulence factors are responsible for *S. epidermidis* pathogenicity, although the ability to produce slime and adhere to prosthetic devices appears to be one major determinant (3, 4).

Several recent studies have suggested that the ability to degrade native elastin molecules may be an important virulence factor for certain bacteria and fungi. In the late 1960s Varadi and Saqueton (13) isolated elastase-producing staphylococci from normal human skin and suggested that these isolates might be the etiologic agent of perifollicular macular atrophy. After this investigation, Murphy and colleagues (2, 11) found a high percentage of elastase-positive CNS in the nares and oral cavities of students and proposed that these strains might invade the crevicular epithelium and play an important role in chronic periodonitis. Since both of these studies pre-dated current taxonomy of CNS and involved isolates from only selected anatomical sites, we investigated the frequency of elastase-producing staphylococci from various clinical sources to determine the potential importance of this enzyme in staphylococcal pathogenesis.

MATERIALS AND METHODS

Strains. A total of 161 isolates belonging to the genus *Staphylococcus* (Gram and catalase positive) were investigated. All isolates originated from clinical material submitted on patients seen at the Mount Sinai Medical Center over a 1-year period. No attempt was made to determine the clinical significance of each isolate. Strains were subsequently divided into two groups (*S. aureus* and CNS) based upon the ability of each isolate to clot rabbit plasma in tube (free coagulase) and slide (bound coagulase) assays. Isolates were identified as *S. aureus* based upon coagulase positivity, production of a beta-hemolysin, and visual pigmentation (yellow to yellow orange) on sheep blood agar.

In addition to these strains, an elastase-positive CNS isolate recovered from the arterial line of a patient was used as a positive control in later experiments. Twenty reference strains, including isolates of *S. saprophyticus*, *S. hominis*, *S. haemolyticus*, *S. cohnii*, *S. warneri*, *S. simulans*, *S. capitis*, *S. xylosus*, *S. hyicus*, and *S. sciuri*, were kindly provided by W. Kloos.

Screening for elastase activity. Since brain heart infusion agar (BHIA; Difco Laboratories, Detroit, Mich.) had been previously suggested to be a suitable basal medium for detecting elastase activity (2), all preliminary assays utilized BHIA supplemented with 0.3% bovine neck ligament elastin (Sigma Chemical Co., St. Louis, Mo.). Overnight cultures of each isolate grown on Mueller-Hinton agar were radially streaked onto elastin agar plates (up to eight strains per plate) and then incubated for 48 h at 37°C. At this time, plates were removed, transferred to room temperature, and incubated for an additional 8 days. On odd days (1 to 9) and on day 10, plates were visually read for evidence of elastase activity indicated by a clearing of the elastin particles peripheral to growth.

The ability of 10 elastase-positive CNS, 10 elastase-negative CNS, and *S. aureus* isolates to produce this exoenzyme on a variety of differential basal media was investigated. Each of these 30 isolates was inoculated in a similar fashion onto veal infusion and heart infusion (Difco), Mueller-Hinton, Trypticase soy, and nutrient agars (BBL Microbiology Systems, Cockeysville, Md.) and onto blood agar base no. 2 (Oxoid Ltd., Basingstoke, England) containing 0.3% elastin. Plates were incubated and read as described previously.

Biochemical identification. All elastase-positive CNS were initially identified to the species level by the minimal criteria suggested for identification of *S. epidermidis* (sensu stricto) by Kloos and Jorgensen (7), which included susceptibility to a 5-μg disk of novobiocin, phosphatase activity, and acid production from D-mannitol, D-trehalose, D-xylose, and sucrose in purple agar base. Strains failing to produce typical biochemical profiles indicative of *S. epidermidis* were further characterized by the 13-test dichotomic scheme of Kloos and Schleifer (8).

RESULTS

Of the 161 staphylococci screened for elastase activity, 24% overall were qualitatively found to produce this enzyme (Table 1). Elastase activity was not detected in any *S. aureus* isolates even after prolonged incubation (10 days), although 41% of the CNS were positive for this trait. The 38 isolates of elastase-producing CNS were recovered from a variety of body sites including wounds (n = 12), blood (n = 9), biopsy material (n = 5), catheters (n = 3), eyes (n = 3), urine (n = 3), and abscesses (n = 2); the source of 1 isolate was unknown. Upon initial screening, elastase activity was detected in over one-half of the 38 isolates only after incubation periods of 5 days or more, suggesting the presence of an

TABLE 1. Frequency of elastase production among staphylococci

Staphylococci	No. of isolates tested	No. elastase positive (%)	Cumulative % positive by day:					
			1	3	5	7	9	10
S. aureus	68	0 (0)	0	0	0	0	0	0
CNS	93	38 (41)	0	34	68	86	97	100

inducible enzyme. When 10 of these strains were replated to BHIA containing elastin, the mean time for detecting elastolytic activity was reduced from 5.2 to 3.1 days.

The basal medium had a pronounced effect upon the ability of staphylococci to produce detectable levels of elastase. Of 10 elastase-positive CNS, all were repeatedly positive when inoculated onto basal BHIA. However, only three of these strains were positive when the basal medium was Trypticase soy and only one isolate was elastolytic on HIA and blood agar base no. 2. All 10 strains failed to produce this enzyme when grown on Mueller-Hinton, veal infusion, and nutrient agars. Elastase-negative *S. aureus* and CNS as determined by preliminary assays failed to elaborate this enzyme when grown on any of the basal media tested.

The species designations for all 38 elastase-positive CNS were determined. Thirty-five isolates were identified as *S. epidermidis* (sensu stricto), being novobiocin susceptible, phosphatase positive, and sucrose positive while failing to produce acid from D-xylose, D-trehalose, and D-mannitol. Three other isolates were subsequently confirmed as *S. epidermidis* (phosphatase negative) by supplementary tests recommended by Kloos and Jorgensen (7). A total of 14 elastase-negative CNS were similarly biotyped with the following results: *S. hominis* ($n = 5$), *S. epidermidis* ($n = 4$, 1 phosphatase negative), and *S. haemolyticus* ($n = 3$); the species status of 2 isolates could not be definitively determined. Of the 20 CNS reference strains tested, all lacked elastolytic activity.

DISCUSSION

Recent studies involving a number of pathogenic microorganisms have indicated that elastase activity may be an important virulence factor in relation to disease-producing potential. In the course of *Pseudomonas aeruginosa* infections, elastase activity has been suggested to promote systemic invasion from localized body sites and to help in the persistence of infection (6, 14). In fungi, elastase-producing strains of *Aspergillus fumigatus* are capable of causing invasive aspergillosis in mice, while elastase-negative isolate are not (9). Among bacteria, elastase activity has also been detected in *Aeromonas* spp., *Vibrio vulnificus*, and *Pseudomonas maltophilia* and has been suggested to possibly play a role in pathogenesis (5, 10). The results of this investigation confirm the findings of previous studies demonstrating a similar activity in the genus *Staphylococcus*.

Elastolytic activity was not detected in any *S. aureus* isolates, results similar to previously published observations (13) but in contrast to the data obtained by Murphy (11). In this latter study, almost 50% of all *S. aureus* strains isolated from the nares or oral cavity of students produced elastase, although over half of these isolates did so only after prolonged incubation, indicating weak activity. The failure of this study to detect a similar activity among *S. aureus* strains may be due to the criteria chosen for elastase positivity (clearing beneath colonies was not considered positive), differences in incubation conditions (Murphy used a 12% CO_2 environment), or the sources of elastin substrate and *S. aureus* isolates. In any instance, elastase activity was more pronounced among CNS, suggesting that these isolates are more efficient producers of this enzyme.

The results of testing isolates for elastase activity under different cultural conditions suggest that this enzyme is inducible in most elastase-positive isolates and that production of this exoenzyme is medium dependent. Elastolytic activity was subsequently found to be associated only with *S. epidermidis* strains (sensu stricto) since no other species or reference strain tested elaborated this enzyme. Since this property was restricted only to *S. epidermidis* strains, the major CNS pathogen, it indirectly suggests that this enzyme may be important in the pathogenesis of this bacterium.

Elastin molecules are proteins found throughout the body in blood vessel endothelium (arteries), lung, and connective tissues. Besides its possible role in skin and periodontal diseases of humans, elastase produced by *S. epidermidis* may facilitate localized multiplication and invasion of tissues containing elastic lamina after colonization (adherence). Further studies based upon the frequency and quantitation of elastase activity from documented *S. epidermidis* infections as opposed to controls will help to determine its diagnostic as well as pathogenic significance.

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