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Serogrouping Single Colonies of Beta-Hemolytic Streptococci with Achromopeptidase Extraction

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Achromopeptidase (TBL-1; Wako Chemicals, USA, Inc., Dallas, Tex.) prepared as a 2,000-U/ml solution will extract the serogroup antigens from single colonies of groups A, B, C, F, and G streptococci in 1 min at room temperature. This enzyme extraction is not effective for the serogrouping of all group D streptococcus species. Achromopeptidase extracts can be used with latex or coagglutination reagents.

Achromopeptidase is a bacteriolytic enzyme derived from *Achromobacter lyticus* (7, 8). This enzyme has been reported to lyse a relatively wide spectrum of bacteria including *Micrococcus*, *Bacillus*, *Sarcina*, *Staphylococcus*, *Streptococcus*, and *Clostridium* spp., as well as some gram-negative bacteria (*Bacteriolytic Enzyme for Biochemical Use: TBL-1*, Wako Chemicals, USA, Inc., Dallas, Tex.). Achromopeptidase has been used for the preparation of bacterial enzymes, DNA, and RNA (7, 8) and for the preparation of protoplasts and spheroplasts (8). The enzyme is now commercially available as a crude product (TBL-1) from Wako Chemicals, USA, Inc. This study demonstrates that achromopeptidase may be used to rapidly extract the specific antigens of groups A, B, C, F, and G streptococci from single colonies grown on sheep blood agar plates.

The streptococci used in this investigation were obtained from stock cultures maintained in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) and 10% glycerol (vol/vol) at −70°C. The serogroups included groups A (15 strains), B (22 strains), C (15 strains), D (11 strains), F (8 strains), and G (10 strains). All of the bacteria used in this investigation were streaked on Columbia sheep blood agar (Scott Laboratories, Inc., Fiskeville, R.I.) and incubated at 35°C in ambient air for 12 to 18 h. Gram staining and the catalase test were done on all of the cultures to presumptively identify them as beta-hemolytic streptococci. The serogroups of the strains grown on the blood agar were confirmed by nitrous acid extraction of single colonies (9) and serogrouping with Streptex reagents (Wellcome Diagnostics, Research Triangle Park, N.C.).

One colony of each strain of streptococcus was placed in 0.20 ml of 0.05 M Tris hydrochloride buffer solution (pH 8.0) containing 50 to 2,000 U of achromopeptidase per ml. The bacterial suspensions were incubated for 1 to 60 min at 37°C. In other experiments, incubations were done at room temperature. Serogrouping of the respective extracts of streptococci was done with Streptex latex reagents (Burroughs Wellcome Co., Research Triangle Park, N.C.) and the Phadebact coagglutination reagents (Pharmacia Diagnostics, Piscataway, N.J.). A drop of each extract was placed on a glass microscope slide and mixed with a drop of the respective serodiagnostic reagent. The reactants were mixed by hand for 1 min to determine the agglutination or coagglutination response of each extract.

Minimal agglutination reactions occurred with all the strains of groups A, B, C, F, and G streptococci after 15 min of extraction at 37°C with 125 U of enzyme per ml. Single colonies of all the strains of groups A, B, C, F, and G streptococci extracted for 15 min at 25°C with 500 to 2,000 U of enzyme per ml yielded relatively strong serogrouping reactions with either the latex or coagglutination serodiagnostic reagents. Furthermore, strong serogrouping reactions of single colonies of groups A, B, C, F, and G streptococci occurred at 1 min when the extraction was done at room temperature with either 1,000 or 2,000 U of enzyme per ml. The respective agglutination and coagglutination reactions were stronger when the extraction solution contained 2,000 U of achromopeptidase per ml. Table 1 gives these data with the effect of concentration and length of incubation of the enzyme on the extraction of single colonies of group A streptococci.

Single colonies, as well as five colonies, and growth from the entire lawn of blood agar plate cultures of four of five strains of *Streptococcus* (*Enterococcus*) *faecalis* extracted with 2,000 U of enzyme per ml for 30 min yielded serogroupable responses with the Phadebact and Streptex group D reagents. The remaining strains of group D streptococci were not detected with the group D serodiagnostic reagents when the strains were extracted with 2,000 U of enzyme per ml for either 30 or 60 min.

No autoagglutination of any bacterial strains occurred when they were placed in Tris hydrochloride containing

TABLE 1. Correlation of achromopeptidase concentration, extraction data, and agglutination reactions of single colonies of group A streptococci with Streptex reagent

Enzyme concn (U/ml)	Temp (°C)	Agglutination reaction[a] at extraction time (min)				
		1	10	15	30	60
2,000	25	4+	4+	4+	4+	4+
	37	4+	4+	4+	4+	4+
1,000	25	3+	4+	4+	4+	4+
	37	4+	4+	4+	4+	4+
500	25	2+	4+	4+	4+	4+
	37	3+	4+	4+	4+	4+
250	25	1+	3+	4+	4+	4+
	37	2+	4+	4+	4+	4+
125	25	0	0	1+	2+	3+
	37	0	1+	2+	3+	4+

[a] Agglutination expressed as a degree of reaction, with clear agglutination equal to 4+ and no agglutination equal to 0.

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2,000 U of enzyme per ml. An achromopeptidase preparation containing 2,000 U of enzyme per ml was stored at 6°C for 4 weeks. Extracts prepared with the 4-week-old enzyme solution yielded agglutination and coagglutination responses similar to that observed with freshly prepared enzyme solution.

Relatively large numbers of beta-hemolytic streptococci and high temperatures are required for extraction of pronase (3) and *Streptomyces albus* lysozyme (2, 6, 10). Heavy metals can inactivate pronase, and chemically clean glassware and double-distilled water must be used in its preparation. Pronase is not effective in the extraction of group D streptococci (4, 5, 10). Group D streptococci may be extracted with mutanolysin only after a 4-h incubation (1). Achromopeptidase, although effective for the extraction of some strains of group D streptococci, would not be acceptable for the serogrouping of that serogroup. The use of this enzyme, therefore, for the serogrouping of beta-hemolytic streptococci colonies associated with throat culture specimens would be most appropriate. Group D streptococci are generally not associated with throat culture specimens and are of little clinical importance when isolated. Unlike mutanolysin extraction (1), achromopeptidase extraction for 1 min at 37°C or at room temperature yielded serogroupable reactions with single colonies of group F streptococci.

Achromopeptidase was similar to mutanolysin in its ability to yield serogroupable extracts with single colonies of beta-hemolytic streptococci. Achromopeptidase, however, can yield serogroupable extracts at room temperature within 1 min, while mutanalysin is used at 37°C after 15 min. Furthermore, mutanolysin has an optimal pH of 6.5, which is associated with autoagglutination responses (1). In contrast, achromopeptidase was used at an optimal pH of 8.0 and was not associated with any autoagglutination responses. Either coagglutination or latex agglutination serodiagnostic reagents may be used with extracts produced by achromopeptidase. In contrast to the results reported with mutanolysin (1), no false-positive test results occurred with achromopeptidase extracts when the Streptex reagents were used.

Unlike mutanolysin, achromopeptidase is now available from a commercial source in this country. Furthermore, a working reagent of achromopeptidase is stable for at least 1 month when maintained at usual refrigeration temperatures. One gram of the crude enzyme costs $73.00, whereas 1 ml of the working reagent containing 2,000 U costs less than $0.01. Accordingly, this enzyme is a cost-effective extraction reagent and could be used instead of nitrous acid for the serogrouping of isolated beta-hemolytic streptococcus colonies from throat cultures (9).

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