STAPHYLOCOCCINS, THEIR PROPERTIES, CLASSIFICATION, AND USE FOR TYPING OF STAPHYLOCOCCI

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The properties of 14 staphylococcins are described and their classification given. Of 721 strains of staphylococci studied, 673 were sensitive to type-specific bacteriocins.

Some strains of staphylococci have the ability to produce antibiotics which have been called staphylococcins [6]. Most workers have investigated the bacteriocinogenic properties of Staphylococcus aureus [2, 5, 11], and data concerning this property in Staphylococcus albus are less frequently found. Staphylococcin A, produced by a nonpathogenic strain of Staphylococcus albus [10], has been investigated in most detail.

In the investigation described below the staphylococcins of staphylococci isolated from healthy human skin were studied.

EXPERIMENTAL METHOD

A modified method of double layers of agar [1] was used to detect bacteriocin production, because of existing data [4] indicating that among the staphylococci of the skin only 30.2% of strains produce bacteriocins resistant to chloroform. The production of staphylococcins in a liquid medium and the study of their properties were carried out by methods described for colicins [3, 7, 8].

EXPERIMENTAL RESULTS

The bacteriocinogenic properties of 721 strains of staphylococci from the Departmental museum, isolated from healthy human skin in 1967-1968, were studied. Experiments showed that 49.6±1.9% of these strains produce bacteriocins. The overwhelming majority of staphylococcins acted on one of the two indicator strains: Staphylococcus albus strain No. 107 or Staphylococcus citreus No. 474. The 14 chloroform-resistant strains producing bacteriocins were divided into two groups by differences in their action on the indicator strains, and by their other properties usually used for the classification of bacteriocins [7, 9] they were additionally divided into nine types (3 types in Group A, 6 types in group B) (Table 1). Seven strains

TABLE 1. Typing of Staphylococci by Sensitivity to Type-Specific Bacteriocins

Type of bacteriocin	No. of sensitive strains
1A	119(16.5±1.4%)
1B, 2B, 3B, 4B, 5B	110(15.2±1.3%)
1B, 2B	50(6.9± .9%)
1A, 5B	29(4.1± .6%)
Remaining types of group B	123(17.1±1.4%)
" " " A + B	242(33.5±1.7%)
Insensitive	48(6.7±0.94%)

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of group A acted only on indicator strain No. 107 and were inactive against strain No. 474; five bacteriocins of type 1A, of which two were produced by Staphylococcus aureus and three by Staphylococcus albus, possessed identical spectra of antibiotic activity, identical morphology of the zone of delayed growth, and gave identical diffusion curves in agar. All produced bacteriocin in liquid medium which withstood heating on a boiling water bath for 1 h in neutral, alkaline, and acid media, none were destroyed by trypsin, and none passed through cellophane or a Seitz filter. Many strains of Staphylococcus albus and potato bacillus were sensitive to bacteriocins of this type. Bacteriocin of type 2A acted only on certain strains, mainly nonpathogenic, of Staphylococcus albus, withstood heating to 56° for 3.5 h, and was not found in the liquid medium. Type 3A bacteriocin acted on strains of Staphylococcus albus and on some strains of Staphylococcus aureus, passed through cellophane, possessed a high rate of diffusion in agar, preserved its activity after heating to 56° for 3.5 h, and was not produced in liquid medium.

Staphylococcins of group B acted on both indicator strains (Nos. 107 and 474). Type 1B bacteriocin possessed a broad spectrum of antibiotic activity: it acted on S. albus, S. citreus, and some strains of S. aureus, Sarcina, and potato and psuedoanthrax bacilli. The bacteriocin passed through cellophane and was found in the supernatant fluid. In its remaining properties it was identical with the type 1A staphylococcins, but was quickly inactivated by heating in an alkaline medium. The type 2B staphylococcins were identical in their properties with staphylococcin 1B but differed much less readily through cellophane and differed in their spectrum of antibiotic activity, for they did not act on S. aureus. Staphylococcin type 3B passed through cellophane, and was partially inactivated at 56° after 1 h. It acted on S. albus, S. citreus, Sarcina, and potato and pseudoanthrax bacilli. Staphylococcins of types 4B and 5B possessed identical physicochemical properties with type 3B bacteriocin, but did not pass through cellophane. They differed in that they inhibited different strains of staphylococci. In addition, a highly characteristic feature of bacteriocin 5B was its distinctive zone of inhibition of growth, in the center of which increased growth of the indicator strain was observed. Type 6B bacteriocin differed by possessing weak activity: it acted only on strains of S. citreus and Sarcina.

Unlike other strains of group B it did not inhibit even indicator strain No. 107. This staphylococcin was thermolabile (inactivated after 1 h at 56°), did not pass through cellophane, and was not produced in a liquid medium.

The spectrum of antibiotic activity of all type-specific staphylococcins was investigated also against other species of microorganisms besides those indicated above. The results showed that Gram-negative bacteria and also strains of yeasts and Candida albicans are insensitive to it.

Sensitivity of all strains (721) of the museum collection was determined against staphylococcins of types 1A, 2A, 1B, 2B, 3B, 4B, and 5B: 673 strains (93.3±0.94%) proved to be sensitive to them. Figures for the bacteriocin types most commonly encountered are given in Table 1. Determination of sensitivity to type-specific bacteriocins can therefore be used as a method of identification of staphylococci.

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