INHIBITORS PRODUCED BY A STRAIN OF *STAPHYLOCOCCUS EPIDERMIDIS* ISOLATED FROM HEALTHY HUMAN SKIN

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The role of "antibiotic" producing strains of bacteria in the microbial ecology of skin is controversial. The potential therapeutic value of these antibiotics has been demonstrated by Selwyn et al (1975) and Gerber and Nowak (1976).

We isolated a strain of *Staphylococcus epidermidis* (Kloos and Schleifer 1975) which inhibited in vitro growth of 4 other bacterial strains isolated from the same skin site. Inhibitor production was stable and not affected by "curing" agents or incubation at 42°C.

Titre was calculated as the reciprocal of the highest dilution producing inhibition on an indicator lawn and was expressed in arbitrary units (AU)ml^{-1}. Maximum titre was obtained by growing the producer strain within Tryptone Soya Agar supplemented with 0.5% yeast extract for 40h at 37°C. Freezing and thawing the agar and centrifuging to deposit cells gave a crude extract (CE) of inhibitor. CE was active against a wide range of Gram positive organisms including *Staphylococcus aureus*, *Bacillus subtilis*, many skin coryneforms and some streptococci; it was inactive against Gram negative strains. Activity was destroyed by trypsin and chymotrypsin. It withstood 80°C for 60 min but not for 90 min and was stable from pH 5.4-8.2. CE adsorbed on to sensitive cells.

CE was partially purified by precipitation with 60% ammonium sulphate. Redissolved product was fractionated on a Sephadex G75 Superfine column calibrated with known molecular weight proteins. The column was eluted with 0.05 M Tris HCl buffer pH 6.0. Fractions revealed two peaks of inhibition corresponding to molecular weights of c. 20,000 and c. 7,000. The higher molecular weight inhibitor (HI) accounted for c. 65% of the total activity but it was unstable. Fractions showed a peak of protease activity coinciding with the elution volume of HI. The instability could be reduced by incubating the producer strain for 24-30h instead of 40h; this gave a CE with lower initial titre but less protease activity. The lower molecular weight inhibitor (LI) was further purified on a Sephadex CM25 cation exchanger column eluted with 0.1M phosphate buffer pH 6.0 and a continuous gradient of 0-0.5M NaCl. LI was eluted as a single peak with 0.11M NaCl. Bacteriostatic and bacteriolytic activity of LI was demonstrated with different indicator strains. Such dual action may be due to autolytic activity of sensitive cells (Tomasz et al, 1970).

TABLE 1: Analysis of 40h (and 24h) yields of inhibitors

Fraction	Total AU inhibitory activity * = titre unstable	Total units protease activity	Percentage yield of inhibitor
CE	12000*(6000)	25540(5400)	
Ammonium sulphate precipitate	5120*(3200)	15840(3170)	42.7(53.3)
HI(G75 peak)	2080*(1440)	11830(2250)	17.3(24.0)
LI(G75 peak)	1120(840)	0(0)	9.3(14.0)
LI(CM25 peak)	768(640)	0(0)	6.4(10.7)

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