SHORT ARTICLES

LIPOLYTIC ACTIVITY OF *STAPHYLOCOCCUS AUREUS* FROM DIFFERENT SOURCES

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The margin of the eyelid is histologically intermediate in position between the mucous membranes and the skin. At this site, staphylococci frequently cause a chronic superficial inflammation that often responds poorly to local antibiotic therapy. The eyelid margin has a fatty layer, secreted by the Meibomian glands in the tarsal plates. If the inflamed eyelid margin is thoroughly defatted, antibiotic therapy is often effective.

There seems to be some relation between the ability of staphylococci to maintain themselves in the eyelid margin and the presence of this fatty layer. Therefore, the lipolytic activity of staphylococci isolated from patients with chronic inflamed eyelid margins and staphylococci isolated from the upper respiratory tract was compared.

MATERIALS AND METHODS

Organisms. A group of 50 strains of *Staphylococcus aureus* isolated from patients with chronic inflamed eyelid margins (ELM strains) was compared with a group of 80 strains of *S. aureus* isolated from the upper respiratory tract (URT strains). All strains were kept for at least six months on meat-infusion agar before being examined.

Measurement of lipolysis. Hydrolysis of lipid substrates was measured by a modification of the method of Smith and Willett (1968). The substrates were triglycerides, containing fatty acids differing in chain length and degree of unsaturation, cottonseed oil (Nutritional Biochemical Corporation, Cleveland, Ohio, USA) and olive oil, which contain different combinations of triglycerides, and the synthetic detergent Tween 80 (Brocades-A.C.F., Holland). These were all prepared as 5% (v/v) emulsions with 3.75% (w/v) gum acacia in phosphate buffer, pH 8.0, and sterilised by flowing steam for 10 min. The stock emulsions were diluted 1 in 10 with medium giving a final concentration of (v/v) substrate 0.5%, and of (w/v) gum acacia 0.375%, peptone 1.2%, NaCl 0.05%, CaCl₂ 0.01%, and Nutrient Agar no. 1 (Oxoid) 1.2%. Basal medium (8.6 ml), consisting of (w/v) peptone 1.2% and Nutrient Agar no. 1 was poured into a petri dish 9 cm in diameter and allowed to solidify. An overlay of medium containing lipid substrate (6.4 ml) was then poured over the basal medium. Diffusion wells were prepared by cutting cylinders of 9 mm diameter in the agar, and 0.1 ml of basal medium was added to each well. The plates were dried for 2 h at room temperature, and the wells were inoculated with 0.03 ml of an 18-h shake culture of the test strain. Each strain was tested in quintuplicate against each substrate. Plates were incubated for 3 days at 37°C, except those containing tricaprln, trilaurin, tripalmitin and Tween 80, which were incubated for 5 days at 37°C. Diameters of the zones of hydrolysis were measured with calipers.

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TABLE
Hydrolysis of various lipid substrates by 50 strains of Staphylococcus aureus isolated from the eyelid margin (ELM) of patients with blepharitis and 80 strains of S. aureus isolated from the upper respiratory tract (URT)

Substrate	Number of carbon atoms in fatty acid	Number of double bonds in fatty acid	Percentage of strains that hydrolysed the substrate		Diameter (mm) of zones of hydrolysis (mean $\pm$ standard deviation) produced by		Difference in mean diameter between ELM and URT strains
			ELM	URT	ELM strains	URT strains	
Tributyrin	4	0	100	100	25.51 $\pm$ 2.47	19.74 $\pm$ 2.50	5.77*
Tricapritin	8	0	100	97.5	23.83 $\pm$ 4.29	20.52 $\pm$ 6.0	3.31*
Tricaprin	10	0	100	100	26.55 $\pm$ 5.22	23.49 $\pm$ 4.77	3.06*
Trilaurin	12	0	100	100	20.69 $\pm$ 3.53	19.66 $\pm$ 3.39	1.03†
Trimyristin	14	0	100	97.5	25.25 $\pm$ 12.74	22.52 $\pm$ 5.16	2.73*
Tripalmitin	16	0	100	100	26.07 $\pm$ 4.45	23.84 $\pm$ 4.44	2.23*
Tristearin	18	0	100	100	20.29 $\pm$ 3.28	17.62 $\pm$ 2.70	2.67*
Triolein	18	1	100	98.75	22.68 $\pm$ 3.24	21.31 $\pm$ 2.53	1.37†
Trilinolein	18	2	100	98.75	17.85 $\pm$ 2.85	16.39 $\pm$ 3.29	1.46*
Trilinolenin	18	3	72	62.5	14.93 $\pm$ 2.31	13.86 $\pm$ 2.45	1.07†
Olive oil§	...	...	100	100	23.55 $\pm$ 4.96	21.79 $\pm$ 4.40	1.76†
Cottonseed oil	...	...	100	97.5	24.71 $\pm$ 4.49	22.15 $\pm$ 5.47	2.56*
Tween 80	...	...	96	95	33.10 $\pm$ 10.09	29.93 $\pm$ 10.69	3.17†

* Significant at the 1% level or less.

† Significant at the 5% level or less.

‡ Not statistically significant.

§ Olive oil consists mainly of triolein.

|| Cottonseed oil consists mainly of trilinolein.

RESULTS

In the table, the average diameters of hydrolysis in mm produced by 50 ELM and 80 URT strains for each substrate are given. Hydrolysis was more pronounced with the ELM than with the URT strains. With the exception of the results obtained with trilaurin, trilinolenin and Tween 80, these differences were statistically significant. As can be seen from the table, a considerable number of both ELM and URT strains did not attack trilinolenin, a triglyceride containing a fatty acid with 18 carbon atoms and 3 double bonds. The number of carbon atoms in each fatty acid residue and the degree of unsaturation is given where possible in the table.

The difference in the average lipolytic activity of the ELM and URT groups of staphylococci for tributyrin, the most discriminating triglyceride, was estimated to be equivalent to 80 mg of pancreatic lipase per 100 ml, on the basis of diffusion tests performed with known amounts of pancreatic lipase.

DISCUSSION

Many bacteria are able to hydrolyse fats that are broken down by bacterial lipases into glycerol and fatty acids or smaller compounds. Cutaneous bacteria are partly responsible for lipase action on the skin, causing the release of fatty acids from hydrolysis of sebum or triglycerides (Strauss and Mescon, 1959).

Lipase production of staphylococci has been investigated since an early date (Orcutt and Howe 1922), and various methods, including the egg-yolk reaction, have been used to study lipase activity. Opalescence around colonies in egg yolk media is caused by lipases (Gillespie and Alder, 1952, Shah and Wilson, 1963 and 1965). Many staphylococcal strains are also able to hydrolyse polyoxyethylene sorbitol compounds, such as Tween 80 (Baird-Parker, 1963, Smith and Willett, 1968),

Lipase production in staphylococci may have some association with pathogenicity. Gillespie and Alder (1952) found that the production of opacity in egg-yolk media was an almost invariable character of strains that caused boils, but that it was absent from type-71 strains that cause superficial vesicular or exfoliative skin lesions (Parker, 1958). In our study we found strains of *S. aureus* from different sources to have significant differences in lipolytic activity for a number of triglycerides and oils.

SUMMARY

The lipolytic activity for a number of triglycerides, oils, and Tween 80 of 50 *Staphylococcus aureus* strains from the margin of the eyelids in patients with chronic blepharitis was found to be significantly greater than that of 80 *S. aureus* strains from the upper respiratory tract.

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