Lipolytic Activity of Human Cutaneous Bacteria

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SUMMARY

Forty-two strains of *Corynebacterium acnes* were compared with 20 strains of staphylococci and 10 of lipophilic diphtheroids for their lipolytic action on 14 substrates and for Tween 80 hydrolysis. All strains were isolated from normal human skin. One lipophilic diphtheroid and 33 *C. acnes* strains hydrolysed tributyrin. Forty-nine bacillary strains hydrolysed Tween 80. None of the other substrates was degraded. *C. xerosis* ATCC 373 and *Propionibacterium acnes* ATCC 11828 acted on tributyrin. *P. acnes* also hydrolysed Tween 80. The staphylococci did not act on Tween 80 but attacked tributyrin, triolein, trilinolenin, trilinolein and oil emulsions. Some cocci acted on tricaprylin, tricaprin and trilaurin; no lipolysis by this group was observed of trimyristin, tripalmitin or tristearin. Pancreatic lipase, tested by the penicylinder diffusion method for comparison, was active on the same substrates, as were the cocci. The addition of glucose, lactate and urea to media did not inhibit lipolysis by the cocci. Sodium oleate inhibited pancreatic lipase action on triolein and cottonseed oil but not on the hydrolysis of tributyrin. Coccal lipase action was not inhibited by oleate. All 74 test strains were uniformly inhibited by neomycin 5-10 µg./ml. Gram-positive cocci rather than *C. acnes* appear primarily responsible for bacterial lipase activity on human skin.

INTRODUCTION

Previous studies indicate that the predominant microbial flora of normal human skin consists of only three groups of bacteria. These include the staphylococci and micrococci, facultative diphtheroids, many of which are lipophilic, and *Corynebacterium acnes* (Marples, 1965). Indirect evidence suggests that cutaneous bacteria are partially responsible for lipase action on skin, causing the release of fatty acids from the hydrolysis of sebum or triglycerides (Strauss & Mescon, 1959). Staphylococci and micrococci produce lipases and many strains hydrolyse polyoxyethylene sorbitan compounds such as Tween 80 (Baird-Parker, 1963). *C. acnes* hydrolyses Tween 80 (Brzin, 1965) and has been reported to act on tributyrin (Puhvel, 1967). Little is known about *C. acnes* action on other lipids or the possible effects of lipophilic diphtheroids on tributyrin or similar substrates. To gain more information about the lipolytic activity of the skin flora, studies were made to compare lipolysis by *C. acnes* strains with other human skin bacteria.

METHODS

Organisms. Cotton-wool swabs were moistened in sterile saline and rubbed over an area of about 4×4 cm. of the faces or forearms of normal human adults; these swabs were discarded. A second swab was rubbed over the same area, placed in 10 ml. sterile saline, and mixed for 30 sec. with an automatic tube shaker. The excess saline was pressed out of the swab into the tube and the swab removed. This saline suspension was used for plating experiments and streaking on the following media: Mannitol salt and Staphylococcus 110 agars (Baltimore Biological Laboratories), Veillonella agar (Difco) and 5 % sheep blood agar with heart infusion base (Difco). One set of these plates was incubated aerobically at 35° for 5 days. A second set including only Veillonella and blood agars was incubated anaerobically in Brewer jars with Gaspaks (BBL) and placed at 35° for 5–7 days. Gram-positive cocci were isolated from mannitol salt or 110 agars. Strains were transferred in brain heart infusion broth (BHI), re-streaked on either selective medium and re-isolated. Cocci were characterized by using the methods of Baird-Parker (1963). Facultative diphtheroids were isolated from blood agar. Isolates of this group were identified by Gram reaction and provisionally as lipophilic strains by their greater growth in BHI broth containing sodium oleate or Tween 80 (Pollock, Wainwright & Mansion, 1949). Efforts to classify this group were attempted by using standard procedures (*Manual of Microbiological Methods*, 1957). *Corynebacterium acnes* strains were isolated from anaerobically incubated plates. They were identified by using current information on the diagnostic characteristics of this organism (Prevot, 1966) and confirmed by agglutination by using the methods and antisera for *C. acnes* of the National Communicable Disease Center, Atlanta, Georgia, U.S.A. Cocci and diphtheroids were maintained on slopes of heart infusion agar containing 0.1 % yeast extract, 0.2 % glucose and 0.2 % Tween 80. *C. acnes* strains were maintained in thioglycollate medium (BBL). *Propionibacterium acnes* ATCC 11828 and *C. xerosis* ATCC 373 were included in the study for comparison.

Measurement of lipolysis. Lipolytic activity was detected by using Spirit blue agar (Difco). Triglycerides (Sigma Chem. Co., St Louis, Mo.), corn oil, olive oil, cholesterol and cottonseed oil (Proflo oil, Traders Oil Mill Co., Fort Worth, Texas) were prepared as 20 % (w/v) emulsions with a 2.5 % sterile solution of gum acacia. Oil emulsions were autoclaved at 118° for 10 min; triglyceride emulsions were sterilized by flowing steam. Substrates were incorporated into the medium in final concentrations of 0.6 % (v/v). Cocci and diphtheroid plates were incubated for 2–5 days. *Corynebacterium acnes* plates were incubated in Brewer jars for 7–8 days. Lipolysis was indicated by clearing of the emulsions. Strains which were negative or weakly positive were examined under a dissecting microscope for evidence of lysis under or at the edge of the colonies.

A crude pancreatic lipase (Nutritional Biochem. Corp., Cleveland, Ohio) was prepared as a 1 % aqueous suspension (pH 8.0) and tested for its activity on the test substrates by filling penicylinders placed in the centre of each plate, and incubated overnight at 35 degrees. On removal of the cylinders, areas of lipolysis were usually 8–10 mm. in diameter.

In later experiments, certain compounds were incorporated into Spirit blue agar to measure their effects on the lipase activity of the bacterial isolates. These compounds

included glucose, sodium lactate, sodium oleate and urea which were sterilized separately by filtration and added to melted media at 46°.

Strains were tested for neomycin sensitivity in the medium of Schultz-Haudt & Scherp (1956). Neomycin solutions were sterilized by filtration. Growth of the strains was measured in a Spectronic 20 colorimeter (Bausch and Lomb). Tween 80 hydrolysis was measured by the method of Wayne (1962).

RESULTS

The Gram-positive cocci were classified as group 2 staphylococci. The lipophilic diphtheroids were Gram-positive catalase-producing bacilli which consistently grew in palisade forms. Glucose and sodium pyruvate were fermented; maltose, sucrose, fructose, mannitol and lactose were not fermented. These strains were also negative in the following tests: urease and indole production, nitrate reduction, gelatin liquefaction, action on litmus milk and motility. Growth occurred in broths containing 6.5 % and 7.5 % NaCl and feebly in 15 % NaCl broth. One strain produced a brown pigment; the other strains were non-pigmented. These strains were tentatively considered related to the *Corynebacteriaceae* because of their morphology and salt tolerance (*Bergey's Manual*, 1957).

Table 1. *Lipolytic activity of selected strains of the predominant human cutaneous flora*

Substrate	Group tested			
	Staphylococci	Lipophilic diphtheroids	<i>Coryne- bacterium acnes</i>	Pancreatic lipase
	Number of positive strains			
Tributyrin	20	1	33	+
Tricaprylin	10	0	0	+
Tricaprin	10	0	0	+
Trilaurin	10	0	0	+
Trimyristin	0	0	0	—
Tripalmitin	0	0	0	—
Tristearin	0	0	0	—
Triolein	20	0	0	+
Trilinolein	19	0	0	+
Trilinolenin	19	0	0	+
Olive oil	19	0	0	+
Cholesterol	0	0	0	—
Cottonseed oil (Proflo)	19	0	0	+
Corn oil	19	0	0	+
Tween 80 hydrolysis	0	9	40	nt*
Total strains tested	20	10	42	

* nt, not tested.

Forty-two strains of *Corynebacterium acnes* were compared with 20 staphylococci and 10 lipophilic diphtheroids for their lipolytic action on 14 lipid substrates and for Tween 80 hydrolysis (Table 1). One lipophilic diphtheroid and 33 *C. acnes* strains hydrolysed tributyrin and 40 strains hydrolysed Tween 80. None of the other substrates was attacked by these two groups. *C. xerosis* ATCC 373 and *Propioni-*

bacterium acnes ATCC 11828 acted on tributyrin. *Propionibacterium acnes* hydrolysed Tween 80. The Gram-positive cocci acted on tributyrin, triolein, trilinolein, trilinolenin and the oil emulsions; only half of the staphylococci attacked tricaprylin, tricaprins and trilaurin and no action was observed by this group on trimyristin, tripalmitin or tristearin. Repeated tests with these three compounds at 0.1% concentrations were negative. Tween 80 was not hydrolysed by the cocci. Pancreatic lipase was active on the same substrates as the lipase-producing cocci.

Lipolytic activity of the cutaneous cocci was also shown by enumerating this group in plating experiments (Table 2). Colony counts of lipolytic bacteria on Spirit blue agar were almost identical to those on mannitol salt agar. Lipolytic colonies from both Spirit blue media showed only Gram-positive cocci; similar results were obtained incubating the plates anaerobically. Lipophilic diphtheroids and *Corynebacterium acnes* were not routinely found among the nonlipolytic colonies developing on Spirit blue media.

Table 2. *Enumeration of the major lipolytic bacteria on human skin*

Medium	Colony counts/sample*
Total	126,000
Mannitol salt agar	85,000
Spirit Blue agar	
+ Tributyrin	85,000
+ Cottonseed oil	83,000

* Counts based on facial swab of 16 cm.² area diluted in sterile saline. Plates were incubated aerobically 5 days at 35°. Total count medium consisted of heart infusion agar with 0.2% glucose, 0.2% Tween 80 and 0.1% yeast extract. Only lipase forming colonies were counted on Spirit Blue agar.

The cocci and pancreatic lipase were retested for lipase action on tributyrin, triolein and cottonseed oil. The following compounds were separately added to each medium: glucose, 2 mg./ml.; sodium lactate, 1 mg./ml.; urea, 0.5 mg./ml.; sodium oleate, 304 µg./ml. (this is equal to a final concentration of 1 µM). Sodium oleate inhibited pancreatic lipase action on triolein and cottonseed oil but not on tributyrin. The other compounds were not inhibitory to pancreatic lipase. The lipolytic action of the cocci was not inhibited by any of the added compounds.

The incorporation of neomycin sulphate 5–10 µg./ml. to broth or agar media inhibited the growth of all strains.

DISCUSSION

Gram-positive cocci appear to be the only major cutaneous bacteria with appreciable lipase activity. These results provide evidence that *Corynebacterium acnes* is not one of the predominant lipolytic bacteria of skin. Inhibition of bacterial lipase in tissue sections treated with neomycin spray (Strauss & Mescon, 1959) is more likely to be due to inhibition of coccal growth and not *C. acnes* growth or activity.

Glucose has been reported to suppress microbial lipase formation (Smith & Alfords, 1966) but the glucose concentration used in our test medium did not prevent the action of the lipolytic cocci. Lactate and urea, which are excreted in large quantities by the skin (Marples, 1965) were also inactive as lipase inhibitors. Certain lipases are known to be inhibited by a concentration of µM-oleate (Smith & Alford, 1966).

This was observed by using pancreatic lipase with a cylinder diffusion method. The accumulation of oleic acid on skin would not appear to inhibit staphylococcal lipase activity. The continued recognition of new lipids secreted by skin (Kellum, 1967*a, b*) may indicate that many more substrates are available for bacteria to act upon. *Corynebacterium acnes*, which resides primarily in the pilosebaceous units of the skin (Marples, 1965) might act differently in response to certain yet unidentified lipids. The C₁₈ unsaturated triglycerides were hydrolysed while failure of the cocci and the pancreatic lipase to act upon trimyristin, tripalmitin and tristearin may be a function of chain length and saturation of the compounds. Variations in qualitative determinations of lipase activity has been reported (Muys & Willemse, 1965). Spirit blue agar was generally satisfactory for this comparative study.

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