

## SHORT REPORTS

### Enzyme Production of Propionibacteria from Patients with Acne vulgaris and Healthy Persons

ULRICH HÖFFLER,<sup>1</sup> MICHAEL GEHSE,<sup>1</sup> MAX GLOOR<sup>2</sup>  
and GERHARD PULVERER<sup>1</sup>

<sup>1</sup>Hygiene-Institut der Universität, Köln, and <sup>2</sup>Hautklinik der Universität, Heidelberg, FRG

Höffler U, Gehse M, Gloor M, Pulverer G. Enzyme production of propionibacteria from patients with acne vulgaris and healthy persons. *Acta Derm Venereol (Stockh)* 1985; 65: 428-432.

375 strains of propionibacteria isolated from pustules, comedones, and from normal skin of patients with acne vulgaris and from healthy persons have been examined for their enzymatic activity. In contrast to healthy individuals, protease and caseinase production of strains from acne patients was significantly lower. On the other hand, DNA'ase production of strains from acne lesions was increased, and lecithinase could be demonstrated in strains from acne patients only. *Key words:* *P. acnes*; *Acne vulgaris*; *Enzymes, extracellular*. (Received February 16, 1985.)

U. Höffler, Hyg.-bakt. Landesuntersuchungsamt "Nordrhein", Auf'm Hennekamp 70, D-4000 Düsseldorf 1, FRG.

The relevance of bacteria in the complex etiology of acne vulgaris has been widely discussed. Some investigators demonstrated differences between acne patients and healthy persons concerning numbers, species and types of propionibacteria (1, 2). Furthermore, differences between various types of propionibacteria are known concerning their metabolic activities. Fanta et al. (3) demonstrated an increased production of porphyrines, and Höffler et al. (4) showed increased production and activity of neuraminidase in strains from patients affected by acne compared with strains from healthy controls. Differences in production of cytotoxin are demonstrable between different comedonal bacteria (5). Whiteside & Voss (6), however, were unable to demonstrate differences in lipolytic activity between strains from acne patients and normal skin.

The aim of this study was to determine whether there are differences in the production of five extracellular enzymes between propionibacteria strains isolated from patients with acne vulgaris and healthy persons.

## MATERIAL AND METHODS

Clinical data of 36 patients with severe acne vulgaris and 26 healthy comparison persons, sampling techniques, and methods for isolation and differentiation of bacteria were described in our previously published studies (7, 8).

### *Bacterial strains*

A total of 375 propionibacteria strains were examined. 104 strains were isolated from nonaffected follicles of acne patients with the cyanoacrylate technique as described earlier (7, 8). 93 strains were from non-inflamed comedones, 107 strains were from acute pustules, and 71 strains were from the pilosebaceous ducts of healthy persons (7).

### *Differentiation of the isolates*

All isolates were classified by standard methods. Further biochemical differentiation was done by using the Minitek differentiation system for anaerobes in GasPak jars (Becton, Dickinson GmbH, Heidelberg, FRG). Biotyping, serological differentiation and phage typing was carried out as previously described (7, 8).

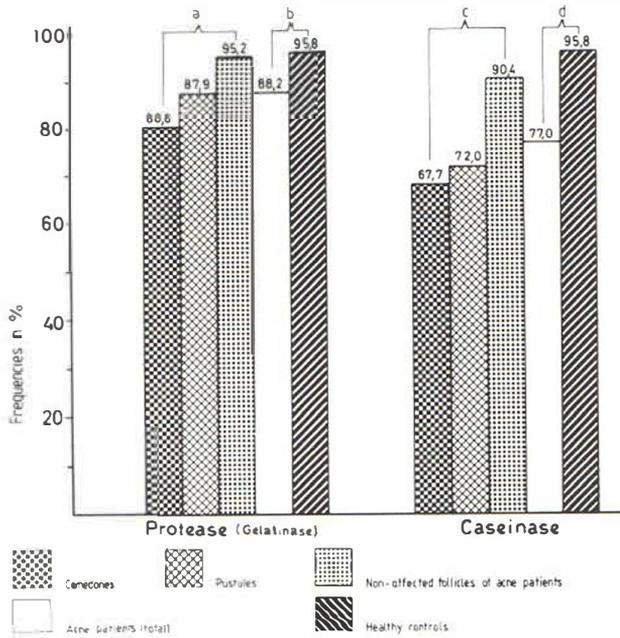


Fig. 1. Production of protease and caseinase by propionibacteria isolated from comedones, pustules, non-affected follicles of acne patients, and from healthy controls.

a=Chi<sup>2</sup>-test:  $p < 0.01$

b=Chi<sup>2</sup>-test: NS

c=Chi<sup>2</sup>-test:  $p < 0.001$

d=Chi<sup>2</sup>-test:  $p < 0.001$ .

### Enzyme tests

The bacteria were tested for the production of five enzymes by use of agar dilution techniques with one loopful of an 48 h old fresh culture of each strain in duplicate. Plates were incubated for 72 h at 37°C by using the Heraeus incubator VT/N<sub>2</sub> (W.C. Heraeus GmbH, D-6450 Hanau, FRG; 85% N<sub>2</sub>, 10% H<sub>2</sub> and 5% CO<sub>2</sub> at normal atmospheric pressure), and the results were read immediately thereafter.

**Protease- (gelatinase-) test.** Production of protease (gelatinase) was tested with agar containing 30 g gelatine per 100 ml (7). After incubation the agar was flowed by sulfosalicylic acid (20% v/v) for 5 min.

**Caseinase test.** The contents of the casein test agar was as follows: Skim-milk powder (L 31; Oxoid Deutschland GmbH, D-4230 Wesel 1), 30 g; Trypton (Difco Laboratories, Michigan, USA), 10 g; Lab-Lemco (Oxoid L 29), 3 g; Yeast-extract (Difco), 1 g; NaH<sub>2</sub>PO<sub>4</sub>, 5 g; agar No. 4 (Oxoid), 14 g; aqua dest., 1 000 ml; pH 7.2. Results were read by observation for growth and clearing of medium.

**Deoxyribonuclease test.** The DNA'ase test agar No. CM 321 from Oxoid was used.

**Lecithinase- (Phospholipase-) test.** Production of lecithinase (phospholipase) was detected by incorporating egg yolk emulsion (10%, v/v) in plates with blood agar base (both from Oxoid) containing 0.6% NaCl.

**Phosphatase test.** The contents of the phosphatase test agar was as follows: Pepton (Difco), 5 g; Lab-Lemco (Oxoid L 29), 5 g; NaCl, 5 g; agar No. 4 (Oxoid), 14 g; aqua dest., 1 000 ml; 10 ml phenolphthaleindiphosphat in aqueous solution (1% v/v). Two different pH values (5.8 and 8.0) were tested.

### Statistical evaluations

Statistical analysis was done in the Rechenzentrum of the University of Cologne by using the Statistical Package for the Social Sciences (SPSS 8). Different groups of strains were paired via chi-square-test with a prescribed significance level of  $\alpha = 0.05$ .

## RESULTS

In Fig. 1 the frequencies of protease (gelatinase) and caseinase production of strains from comedones, pustules, nonaffected follicles of acne patients and healthy controls are shown. Strains of healthy controls and unaffected skin of acne patients showed high

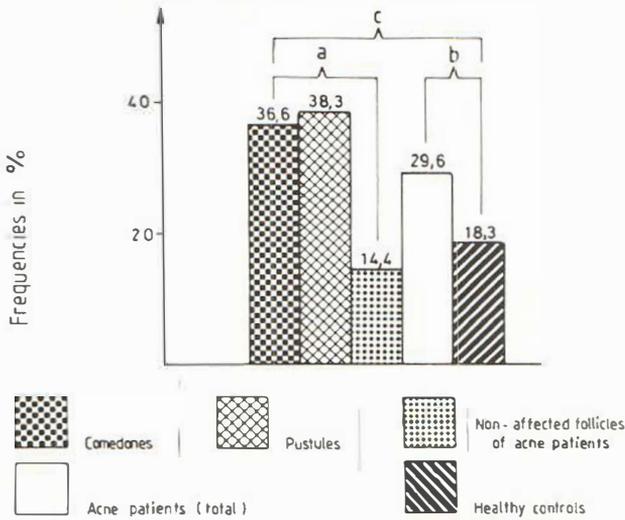


Fig. 2. Production of deoxyribonuclease (DNA'ase) by propionibacteria isolated from comedones, pustules, non-affected follicles of acne patients, and from healthy controls.  
 $a = \text{Chi}^2\text{-test: } p < 0.001$   
 $b = \text{Chi}^2\text{-test: NS}$   
 $c = \text{Chi}^2\text{-test: } p < 0.01$ .

frequencies of protease production, whereas strains of acne comedones were significantly less active ( $\alpha = 0.0032$ ). In contrast to healthy individuals, caseinase activity of strains from acne patients is significantly lower ( $\alpha = 0.0005$ ). Considering different lesions of acne patients it is evident that strains from nonaffected skin produce caseinase as often as strains from healthy individuals, but strains from comedones and pustules are significantly less active ( $\alpha = 0.0002$ ).

Fig. 2 demonstrates the frequencies of DNA'ase production of different strains. The comparison between healthy controls and acne patients shows a distinct increase. All tested *P. granulosum* strains produced DNA'ase, but only 11.7% of the *P. acnes* strains.

As to be seen in Fig. 3, lecithinase could not be demonstrated in strains from healthy individuals, but in 17.4% of all strains from acne patients.

Fig. 4 shows the frequency of phosphatase production. No significant differences could be shown between different groups of strains; values differed from 88.7% (controls) to 96.8% (comedones).

## DISCUSSION

Only little information about the production of enzymes by propionibacteria isolated from acne lesions and from healthy human skin was found in previous publications. Differences in lipase activity (6) and hyaluronate lyase (9) between strains from different origin could

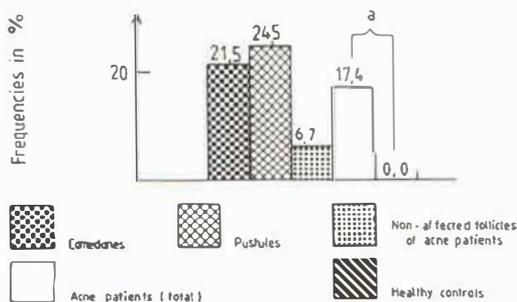


Fig. 3. Production of lecithinase (phospholipase D) by propionibacteria isolated from comedones, pustules, non-affected follicles of acne patients, and from healthy controls.  
 $a = \text{Chi}^2\text{-test: } p < 0.001$ .

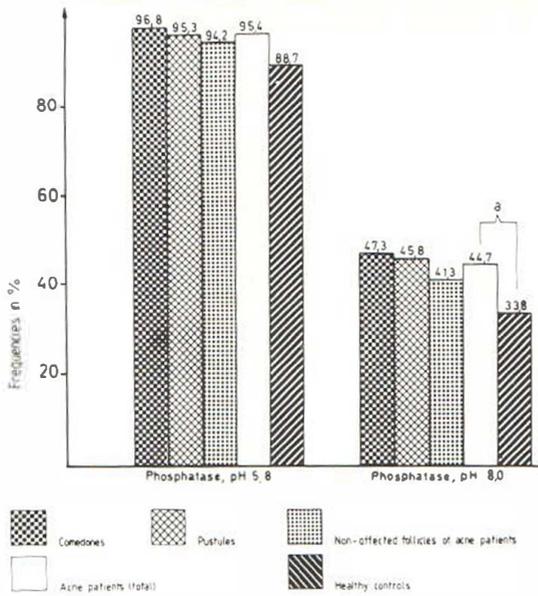


Fig. 4. Production of phosphatase by propionibacteria isolated from comedones, pustules, non-affected follicles of acne patients, and from healthy controls.

$a = \text{Chi}^2\text{-test: NS.}$

not be demonstrated. However, neuraminidase activity could be discovered in a higher percentage of *P. acnes* strains isolated from acne lesions compared with strains from normal human skin (4). Neuraminidase-positive strains, moreover, produced statistically significantly higher amounts of the enzyme than isolates from normal skin (4).

The results of our study with 375 strains of propionibacteria indicate that isolates from healthy controls and the unaffected skin of acne patients are more often proteolytic active than isolates from comedones and pustules. On the other hand, strains from nonaffected skin are lower producers of DNA'ase and lecithinase than strains from acne lesions. These two latter enzymes were almost exclusively produced by the species *P. granulosum*, which in a former study (7) could be differentiated in acne patients only and was more frequently found in acne lesions than in the unaffected follicles of acne patients. By splitting cellular tissue detritus and skin surface lipids, it seems possible that these two enzymes of propionibacteria act as etiological factors in the complex pathogenesis of acne vulgaris.

#### ACKNOWLEDGEMENT

This study was supported by research grants of the Deutsche Forschungsgemeinschaft.

#### REFERENCES

- Lentze I, Ko HL, Höfller U. Differenzierung unterschiedlicher Propionibakterien-Spezies aus Acne vulgaris - Effloreszenzen. *Hautarzt* 1979; 30: 242-247.
- Leyden JJ, McGinley KJ, Mills OH, Kligman AM. Propionibacterium levels in patients with and without acne vulgaris. *J Invest Dermatol* 1975; 65: 382-384.
- Fanta D, Formanek I, Poitschek CH, Thurner J. Die Porphyrinproduktion des Propionibacterium acnes bei Acne und Seborrhoe. *Arch Dermatol Res* 1978; 261: 175-179.
- Höfller U, Gloor M, Nicolai Hv. Neuraminidase production by Propionibacterium acnes-strains isolated from patients with acne vulgaris, seborrheic eczema and healthy subjects. *Zentralbl Bakteriol Abt I [Orig A]* 1981; 250: 122-126.

5. Puhvel SM, Sakamoto M. Cytotoxin production by comedonal bacteria (*P. acnes*, *P. granulosum*, *S. epidermidis*). *J Invest Dermatol* 1980; 74: 36-39.
6. Whiteside JA, Voss JG. Incidence of lipolytic activity of *Propionibacterium acnes* (*Corynebacterium acnes* group I) and *P. granulosum* (*C. acnes* group II) in acne and in normal skin. *J Invest Dermatol* 1973; 60: 94-97.
7. Gehse M, Höffler U, Gloor M, Pulverer G. Propionibacteria in patients with acne vulgaris and in healthy controls. *Arch Dermatol Res* 1983; 275: 100-104.
8. Höffler U, Gloor M, Peters G, Ko HL, Bräutigam A, Thurn A, Pulverer G. Qualitative and quantitative investigations on the resident bacterial skinflora in healthy persons and in the non-affected skin of patients with seborrheic eczema. *Arch Dermatol Res* 1980; 268: 297-312.
9. Höffler U. Production of hyaluronidase by propionibacteria from different origins. *Zentralbl Bakteriol Abt I [Orig A]* 1979; 245: 123-129.