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ENZYME PRODUCTION AND ANTIBIOTIC SUSCEPTIBILITY OF *PROPIONIBACTERIUM ACNES* AND *P. GRANULOSUM* FROM ACNE VULGARIS PATIENTS AND HEALTHY PERSONS

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القابلية لإنتاج الأنزيمات والحساسية للمضادات الحيوية في زيباكنزيم أسينس وبروبيو نيباكنزيم قراتيولوزم المعزولة من المصابين بحب الشباب والأشخاص الأصحاء

عـزلت سـبعة عشـرة سـلالة من *Propionibacterium acnes* وعشرة سلالات من *P. granulosum* من المرضى المصابين بحب الشباب كما عزلت عشرة سلالات من كل نوع من الأشخاص الأصحاء. فحصت جميع العزلات لمعرفة قابليتها لإنتاج الأنزيمات وحساسيتها تجاه عشرة مضادات حيوية. كانت النسبة المئوية لإنتاج أنزيمات اللاييز واللسثتيز والكازينيز لسلالات كل النوعين من لطخة حب الشباب أعلى من تلك المعزولة من الأشخاص الأصحاء. وهذا عكس ما وجد بالنسبة لأنزيم الجيلاتينيز. أنتج أنزيم الهيمو لايسين من قبل جميع السلالات تقريبا، أظهرت جميع العزلات مقاومة تجاه الأمبسيلين والبنسلين والتتراسايكلين وكانت سلالات كلا النوعين المعزولة من الأشخاصالأصحاء أكثر حساسية لبقية المضادات الحيوية من تلك المعزولة من لطخة حب الشباب بفروق معنوية عند مستويات مختلفة .

ABSTRACT

Seventeen strains of *Propionibacterium acnes* and ten strains of *P. granulosum* were isolated from acne patients, in addition to ten isolates of each species from healthy persons. All strains were examined for their enzymatic activity and antibiotic susceptibility to ten antibiotics. Percentage of lipase, lecithinase and casenase production by strains of both species from acne lesions was higher than that from healthy persons in contrast to gelatinase. Haemolysin was produced almost by all strains.

All isolates were resistant to ampicillin, penicillin and tetracycline. Strains of both species from healthy persons were more susceptible to the remaining antibiotics than those from acne lesions with significant differences at various levels.

INTRODUCTION

The relevance of *Propionibacterium* in the complex etiology of acne has been widely discussed. Some investigators demonstrated differences between acne patients and healthy persons concerning number, regional variations, species and types of *Propionibacterium* (1, 2, 3, 4). Their role in the genesis of fatty acids has also been reported (5, 6, 7, 8). The effect of glucose concentration, oxygen tension and pH on growth rate and extracellular enzyme production was also examined (9, 10, 11). Extracellular enzymes may function to supply the organism with carbon/energy sources in vivo (7, 12). Hoeffler et al. (13) were the first to study the difference in the production of extracellular enzymes between propionibacteria strains isolated from acne patients and healthy persons. However, different species of *Propionibacterium* were not considered in their study.

The aim of this study was to determine and compare the ability of two species of *Propionibacterium: P. acnes* and *P. granulosum* to produce five extracellular enzymes and the susceptibility of both species towards ten antibiotics. Strains of the two species were isolated from acne patients and healthy persons.

MATERIALS AND METHODS

Samples were obtained from comedons (previously wiped with 70% alcohol) of the forehead and cheeks of acne patients, age ranged between 14-24 years, by comedon extractor (7). Sterile cotton swabs dipped in sterile brain heart infusion broth were used to obtain samples by rotating the swab on the skin of healthy person (without prior sterilizations). Samples were cultured immediately on brain heart infusion agar and incubated anaerobically in Gas Pack Jars for 6 days at 37°C. Colonies showing characteristics of *Propionibacterum* were Gram stained and examined for morphological, physiological and biochemical characteristics. Species were differentiated according to standard methods (14, 15).

In the present investigation, seventeen strains of *P. acnes* and ten strains of *P. granulosum* from acne patients (A strains) and ten isolates of each species from healthy persons (H strains) were selected randomly.

Samples were taken from subjects who did receive neither topical nor oral antibiotics for at least one month prior to sampling.

Enzyme tests

The selected strains were tested for the production of lipase using Tween 80, and gelatinase (protease) (14), lecithinase (phospholipase) using egg yolk emulsion (16), casinase using litmus milk agar and haemolysin using sheep blood agar (15). One loopful of fresh cultures of each strain was cultured in triplicate. Plates were incubated for up to 6 days anaerobically at 37°C.

Antibiotic susceptibility

The method of Piddock (17) was adopted to test antibiotic susceptibility by disk diffusion method using Muller-Hinton agar. The following concentrations (µg per disk) of the antibiotics (Oxoid) were used: ampicillin, 10; chloramphenicol, 30; clindamycin, 10; erythromycin, 15; gentamycin, 10; kanamycin, 30; neomycin, 30; penicillin, 10 units; streptomycin, 10 and tetracycline, 30. The plates were incubated anaerobically at 37°C for up to six days. The mean zone diameter of inhibition of five isolates of each species from the acne patients and healthy persons was considered for comparison, susceptibility was estimated according to Difco (18). The strains were coded as resistant or susceptible with intermediate strains being included in the resistant class.

RESULTS

Enzyme production

Percentage frequencies of strains producing lipase, lecithinase, casinase, gelatinase and haemolysin of A and H strains of *P. acnes* and *P. granulosum* are shown in Fig. (1).

No significant difference was found between the ability of strains to produce lipase, neither between the two species nor between A and H strains. The ability of strains to produce lecithinase was much lower than that of lipase where the highest frequency for production was only 40% recorded by A strains of *P. granulosum* and none of H strains produced this enzyme. Nevertheless, a significant difference ($P<0.05$) was detected between A and H strains of *P. acnes*. A strains of both species had approximately similar percentage of casinase production and it is significantly higher than that of H strains ($P<0.05$). In contrast, H strains of both species showed higher percentage in gelatinase production than A strains. However, A and H strains of *P. acnes* had higher ability than that of *P. granulosum*. Almost all strains (A and H) of both species produced haemolysin.

Antibiotic susceptibility

Results of antibiotic susceptibility are shown in Fig. (2). Ampicillin, penicillin and tetracycline exerted weak effects on both species. However, A strains of both species showed higher resistance. Resistance was significant ($P<0.05$) between A and H strains of *P. acnes* towards ampicillin and tetracycline.

Although strains of both species were sensitive to the remaining seven antibiotics, H strains, however, were the most sensitive. Significant differences were detected between A and H strains of *P. acnes* towards streptomycin ($P<0.01$), erythromycin and gentamycin ($P<0.1$). A and H strains of *P. granulosum* were significantly different in their susceptibility towards gentamycin, kanamycin and streptomycin ($P<0.1$).

DISCUSSION

Enzyme production

Only little information about the production of enzymes by *Propionibacterium* isolated from acne lesions and healthy normal skins was found in previous publications. Hoeffler *et al.* (13) was the first to demonstrate differences in proteolytic activity, DNase and lecithinase by strains of *Propionibacterium* isolated from these two sites but with no consideration to the variations between species. The results of our study with A and H strains of the two species indicate approximately equal ability of A strains of both species for production of lipase, casinase and lecithinase (Fig. 1). However, Whiteside and Voss (19) reported higher levels of lipase production by *P. granulosum* than *P. acnes*. Percentage production of these enzymes was as well, higher by A strains of both species as compared with H strains. This agrees with the suggestion of Ingram et al. (10) that

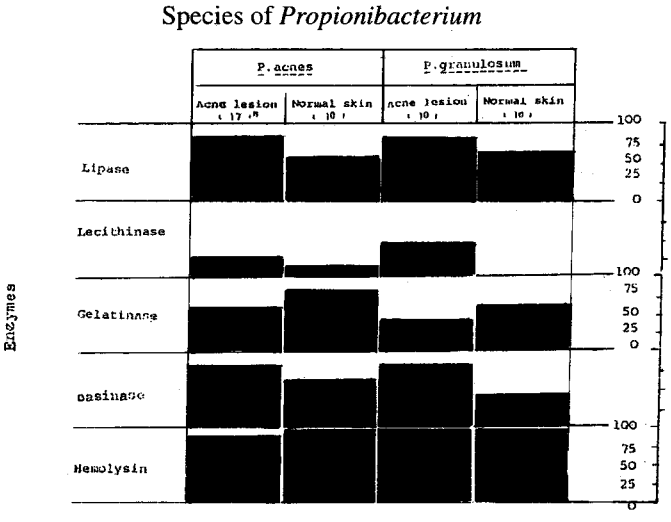

Fig. 1: Percentage frequency of extracellular enzymes produced by *Propionibacterium acnes* and *P. granulosum* isolated from acne lesion and normal skin. n = No. of isolates.

extracellular enzymes in addition to their function in nutrition, they are potentially important with regard to complement activation, to be antigenic or release of chemotactic substance in vivo. These factors play an important role in causing inflammation in acne (7, 9). Furthermore, Holland *et al.* (20) indicated that microenvironments may reflect the physiological response of the bacteria.

The higher level of proteolytic activity (gelatinase) demonstrated by H strains of *P. acnes* and *P. granulosum* (80 and 60% respectively) as compared with A strains (58.8 and 40% respectively) comes along with the reports of Holland et al. (7) and Bladon *et al.* (21) who noted the importance of protease in the degradation of keratin to maintain the natural activities of sebaceous follicles.

It seems possible, therefore, that these two species act similarly and are relevant as etiological factors in the pathogenesis of acne vulgaris.

Antibiotic susceptibility

When antibiotic resistance pattern of A and H strain of both species were compared (Fig. 2), it was found that they were correlated with each other. However, A strains showed higher resistance which might reflect the misuse of antibiotics. Tetracycline, erythromycin and clindamycin are the drugs of choice as effective and relatively free of side effects (22). But unfortunately, microbial resistance towards tetracycline however, develops in 20-25% of patients after 2 months or 2 years of therapy with topical antibiotic (23). Eady et al. (24) reported that overgrowth of tetracycline resistant organisms occurs so quickly because of the high level of resistant microorganisms to this antibiotic before treatment. Such finding agrees with the result of the present study. Nevertheless, our isolates were susceptible to erythromycin and clindamycin; the latter has proved to be an effective alternative to tetracycline (23, 24, 25).

In addition to tetracycline, A and H strains of both species

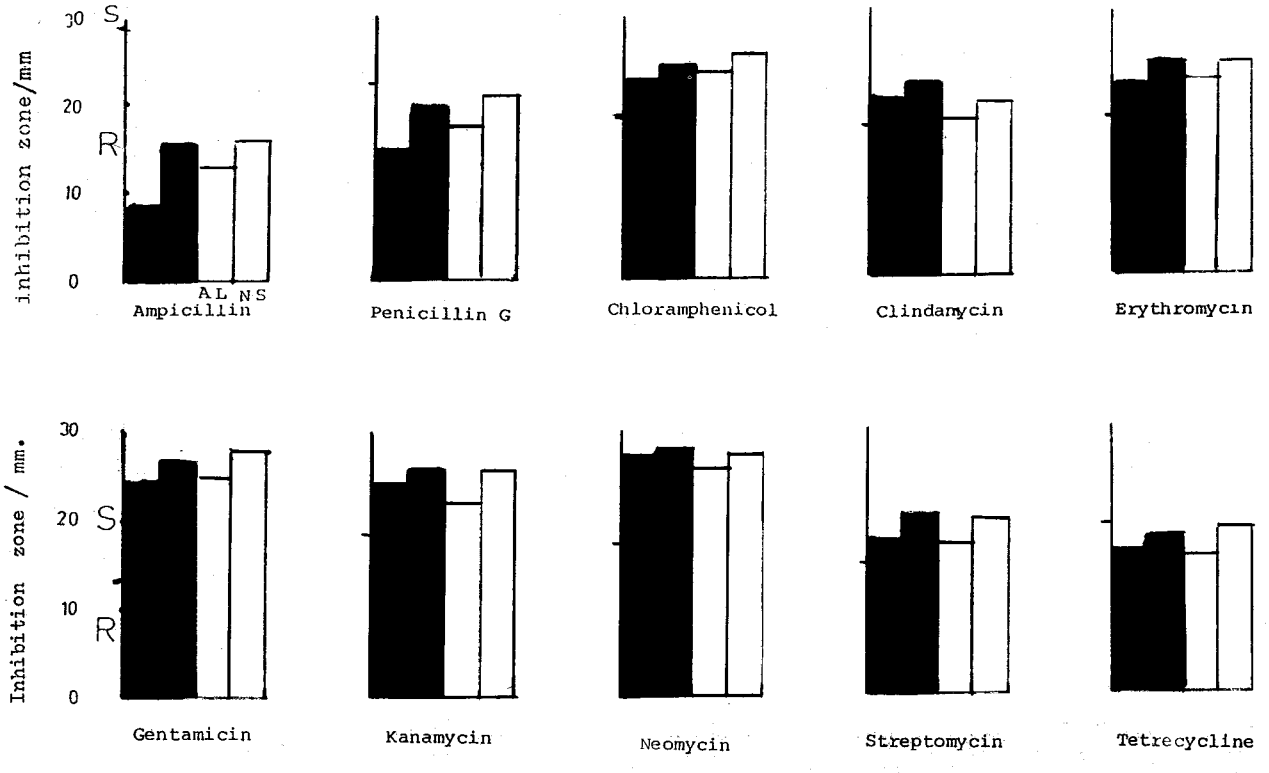

Fig. 2: Sensitivity of *Propionibacterium* acnes and *P. granulosum* isolated from acne lesion and normal skin towards ten antibiotics.

were also found resistant to ampicillin and penicillin. Resistance might be referred to the lack of permeability to the drug (26), production of the enzyme β-lactamase (27) and enhanced virulence or increased ability to disseminate (20). The emergence of antimicrobial resistance is of great clinical and economic importance as multiple-resistant bacteria are becoming increasingly implicated in the infections of compromised hosts.

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