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Biofilm formation by *Propionibacterium acnes* is associated with increased resistance to antimicrobial agents and increased production of putative virulence factors

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Abstract

Propionibacterium acnes plays an important role in the pathogenesis of acne vulgaris, a common disorder of the pilosebaceous follicles. Recently, it was suggested that *P. acnes* cells residing within the follicles grow as a biofilm. In the present study, we tested the biofilm-forming ability of several *P. acnes* strains in a microtiter plate model. We also evaluated the resistance of biofilm-grown *P. acnes* towards antimicrobial agents commonly used in the treatment of acne and the production of putative virulence factors. Our results indicate that *P. acnes* can form biofilms in vitro. The results also show that sessile *P. acnes* cells are more resistant to various commonly used antimicrobial agents than planktonic cells. In addition, sessile cells produce more extracellular lipases as well as significant amounts of the quorum-sensing molecule autoinducer-2.

Keywords: *Propionibacterium acnes*; Biofilm; Acne vulgaris; Lipase; Quorum sensing; Antibiotic resistance

1. Introduction

Acne vulgaris is a common multifactorial disorder of the pilosebaceous follicles, involving sebaceous hyperplasia, follicular hyperkeratinization, hormone imbalance, bacterial infection and immune hypersensitivity [14,18,42,45]. Part of the inflammation seen in acne is the result of the host response to *Propionibacterium acnes*. As the organism metabolizes sebaceous triglycerides, it releases free fatty acids that irritate the follicular wall and surrounding dermis. In addition, *P. acnes* also produces exoenzymes and neutrophil chemoattractants [19,42,45]. Besides keratinolytic and sebosuppressive agents like retinoids [26], antibacterial agents are an important part of the treatment of acne. Topical antimicrobial agents include benzoyl peroxide, clindamycin, erythromycin, tetracycline, azelaic acid, triclosan and various combinations of these agents [1,11,21,23,40,41,44]. In cases where topical treatment is not succesful or in patients at risk for scarring of the skin and pigmentary changes, systemic antibiotics are indicated. These include tetracycline, doxycycline, minocycline and erythromycin [31]. However, data from several studies indicate a drastic increase in the proportion of patients carrying *P. acnes* strains resistant to one or more antibiotics [7,8,27,28,30,31]. The relationship between skin colonization with resistant *P. acnes* isolates and treatment outcome is complex and the clinical significance of resistance is not always entirely clear [12]. Nevertheless, careful analysis of clinical trials performed over the last two decades showed that there has been a gradual decrease in the efficacy of topical erythromycin, most likely related to the development of resistance [34].

It is well established that bacterial biofilms play an important role in the pathogenesis of many human infections [9,10,29]. One of the striking properties of sessile cells (i.e. cells growing in a biofilm) is their increased resistance to antimicrobial agents [24,37]. Factors considered to be responsible

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for this phenotype include restricted penetration of antimicrobials, decreased growth rate, expression of resistance genes and the presence of resistant ''persister'' cells [20,24,37].

Recently, Burkhart and Burkhart [3–5] suggested that *P. acnes* cells residing within the pilosebaceous unit grow as a biofilm. However, conflicting data on the biofilm-forming ability of *P. acnes* have been published. Ramage et al. [30] showed that *P. acnes* strains can form highly resistant biofilms on various biomaterials. In contrast, Takemura et al. [39] showed the inability of *P. acnes* to form biofilms on dental root filling gutta-percha points. Interestingly, the recently published genome sequence of *P. acnes* [2] shows that the organism has three separate clusters of genes that encode enzymes involved in extracellular polysaccharide biosynthesis, suggesting that it is capable of forming the necessary extracellular biofilm matrix. Several proteins that may be involved in adhesion were also identified [2,5].

In the present study, we investigated the biofilm-forming ability of *P. acnes* in vitro. Subsequently, we evaluated the resistance of biofilm-grown *P. acnes* towards antimicrobial agents commonly used in the topical and/or systemic treatment of acne and determined whether or not differences between planktonic and sessile cells could be observed in terms of production of putative virulence factors, including lipases and the quorum-sensing molecule autoinducer-2 (AI-2).

2. Materials and methods

2.1. Strains and culture conditions

The following *P. acnes* strains were used: LMG 16711 (ATCC 6919) (isolated from human facial acne in the UK), LMG 16712 (CCUG 10171) (isolated from human acne), LMG 16714 (CCUG 33192) (isolated from human finger wound caused by fish bone) and LMG 16715 (CCUG 33206) (isolated from human blood). All strains were obtained from the BCCM/LMG Bacteria Collection (Ghent, Belgium) and were cultured on reinforced clostridial medium (RCM) (Oxoid CM0149B) (Oxoid). Plates were incubated at 37 °C under anaerobic conditions using the Anaerocult A Mini system (Merck).

2.2. Testing biofilm-forming ability

In order to determine whether *P. acnes* strains could form biofilms, and to determine the kinetics of biofilm growth, we used a previously described microtiter plate test [36], with modifications. Sterile polystyrene round-bottomed 96-well plates (TPP) were inoculated with 100 µl of a culture of *P. acnes* of OD_{600} appr. 0.1 and incubated anaerobically at 37 °C. Following 4 or 24 h of adhesion, the supernatant containing planktonic cells was removed and the plates were washed with 100 µl sterile phosphate-buffered saline (PBS) (0.14 M NaCl, 0.003 M KCl, 0.003 M $Na_2HPO_4.12H_2O$ and 0.0015 M KH_2PO_4). Subsequently, 100 µl fresh RCM was added and the plates were further incubated for 24 or 48 h. Following incubation, the supernatant was again removed and plates were washed with 100 µl sterile PBS. For quantification by plating, all biofilm material was removed from the wells by repeated and extensive rinsing with 0.9% NaCl, and serial dilutions were made on RCM. For crystal violet staining, 100 µl 99% methanol (Sigma) (15 min) was used for fixation of the remaining adherent cells, after which the supernatant was removed and the plates were air-dried. Then 100 µl 0.5% crystal violet solution (Pro-Lab Diagnostics) was added to the plates. After 20 min at room temperature, the excess crystal violet was removed and the plates were washed by placing them under running tap water. Excess water was removed and the plates were air-dried. Subsequently, the crystal violet dye bound to adherent cells was released by adding 160 µl 33% acetic acid (Acros) and the optical density was measured at 590 nm, using a multilabel microtiter plate reader (Wallac Victor^2, Perkin Elmer). For determination of the fraction of living cells in the biofilm, the presence of metabolically active sessile cells was detected with resazurin. Resazurin is a viability stain that depends on the presence of metabolic activity and will only stain viable cells. For this assay, we first rinsed the plates to remove planktonic cells; subsequently we added 100 µl of sterile 0.9% NaCl and 20 µl of CellTiter-Blue (Promega). Plates were incubated aerobically for 1 h at 37 °C. Fluorescence (excitation wavelength 535 nm, emission wavelength 590 nm) was measured using a multilabel microtiter plate reader (Wallac Victor^2) (Perkin Elmer). In order to observe biofilm morphology, biofilms were allowed to form in 55 mm polystyrene Petri dishes. A similar approach to that described above for the microtiter plates was followed. Two ml of an overnight culture was added to a 55 mm Petri dish. Following 24 h of adhesion, the supernatant was removed and the dish was washed with 3 ml sterile PBS. Two ml fresh RCM were added, and the plates were further incubated for 24 or 48 h. After incubation, the supernatant was removed, the dish was washed with 3 ml sterile PBS and 2 ml 1/200 dilution of the fluorescent stain SYTO9 (Molecular Probes) in 0.9% NaCl was added to the dish. Following incubation (at room temperature in the dark) for 15 min, biofilm structures were visualized by epifluorescence microscopy using an Olympus BX41 microscope equipped with an Olympus U-RFL-T laser (Olympus GmbH).

2.3. Biofilm development in the presence of antimicrobial agents

The antimicrobial agents included in this study were selected because they are often used in the treatment of acne (e.g. erythromycin, benzoyl peroxide), have been proposed as alternative anti-acne treatment (e.g. triclosan) or have been suggested to have an antibiofilm effect (e.g. salicylic acid). Concentrations were selected to represent actual therapeutic concentrations. Growth in the presence of the following agents was tested (all percentages are w/v): 0.5% erythromycin, 2% salicylic acid, 0.3% doxycycline, 30 mM azelaic acid, 0.5% minocycline, 0.5% oxytetracycline, 1% clindamycin, 0.1% triclosan, and 2.5% and 5% benzoyl peroxide. In addition, we also tested growth in the presence of the combinations 5% benzoyl peroxide +0.5% erythromycin, and 5% benzoyl peroxide +1%

clindamycin. Antimicrobial agents were dissolved directly in RCM (for 0.5% erythromycin and 2% salicylic acid), or in dimethylsulfoxide (DMSO) or (in the case of benzoyl peroxide) acetone. We determined the *P. acnes* LMG 16711 biofilm biomass in the presence of antimicrobial agents by culturing the biofilms in 96-well plates, as described above. However, following adhesion and a 24 h period during which the biofilm could develop, the plates were washed and 100 µl RCM containing one or more antimicrobial agents was added. Subsequently, the plates were further incubated for 24 h. Each plate contained at least 12 wells with RCM without any antimicrobial agent (positive control) and at least 12 wells with uninoculated RCM (negative control). In order to establish the effect of the antimicrobial agent(s) on the biofilm, biofilms were stained with crystal violet or the CellTiter Blue kit as described above.

2.4. Detection of putative virulence factors

Sterile supernatants of planktonic and sessile *P. acnes* cells (LMG 16711, LMG 16712 and LMG 16715) were prepared by filtering bacterial suspensions through 0.22 µm membranes (Millipore) and assayed for the presence of lipase, phospholipase, protease, DNAse and hemolytic activity. Extracellular lipase activity was determined by using 4-methylumbelliferyl (4-MU)-based fluorogenic substrates, as described previously [13]. In the present study we used 4-MU palmitate, 4-MU stearate and 4-MU oleate (Molecular Probes). Briefly, 200 µl of the substrate (0.2 mg/ml DMSO) was mixed with 200 µl sterile supernatant in black 96-well microtiter plates (CulturPlate-96F, Packard Biosciences) and incubated at 37 °C; at regular time intervals fluorescence was measured using a multilabel microtiter plate reader (Wallac Victor^2) (Perkin Elmer) (excitation wavelength 355 nm, emission wavelength 460 nm). The activity of phospholipase C (phosphatidylcholine cholinephosphohydrolase) was determined using *p*-nitrophenylphosphorylcholine as substrate, as described previously [33].

Extracellular protease activity was assayed by: (i) adding sterile supernatant to RCM plates containing 3% skim milk powder (Oxoid); and (ii) the azocaseine method [26]. DNase activity was measured by adding sterile supernatants to DNase medium (Oxoid). Similarly, hemolytic activity was assayed by adding sterile supernatant to RCM containing 5% horse blood.

2.5. Detection of AI-2 in supernatant of P. acnes planktonic and sessile cells

The amount of AI-2 present in the filter-sterilized supernatant of planktonic and sessile *P. acnes* LMG 16711 cells was quantified using *Vibrio harveyi* biosensor strains, as described previously [16]. Sequence alignment of LuxS proteins was performed using ClustalX software [43].

2.6. Statistical analyses

One-tailed *t*-tests were performed using the SPSS 12.0 software package (SPPS Inc).

3. Results and discussion

3.1. Biofilm-forming ability of P. acnes isolates

In a first set of experiments, we determined the optimal conditions for *P. acnes* biofilm formation by varying: (i) the density of the inoculum; (ii) the adhesion time; and (iii) the biofilm growth time. Under the conditions used, a mature biofilm (i.e. a biofilm in which the biomass does not significantly increase any further) was established following 24–48 h of incubation, irrespective of adhesion time (data not shown). This absence of a further increase may be due to exhaustion of nutrients and/or reduced contact between biofilm cells and available nutrients. Following a thorough evaluation of all parameters, we decided to use planktonic cultures 60–70 h old as inoculum, an adhesion time of 4 h and a biofilm growth time of 24 h. This combination of parameters was the most practical and resulted in biofilms with sufficient biomass (Fig. 1).

Using these parameters, biofilms could be obtained for *P. acnes* LMG 16711, LMG 16712 and LMG 16715 (Fig. 1). However, *P. acnes* LMG 16714 did not form a biofilm

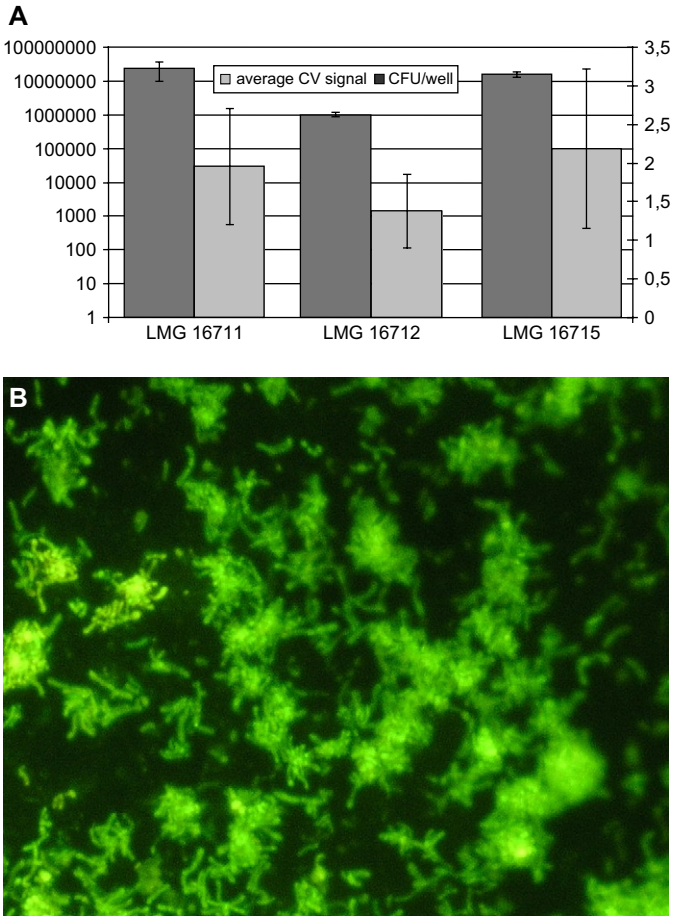

Fig. 1. A. Number of CFUs recovered per well (left Y-axis) and crystal violet absorption (right Y-axis) for different *P. acnes* biofilms. B. Visualization of early stages (24 h) of biofilm formation of *P. acnes* LMG 16711, using SYTO9 and epifluorescence microscopy (magnification: 500×).

in our system, as no biofilm was visible in the wells of the microtiter plate and no significant staining with crystal violet was observed. The number of CFUs present in the biofilm was determined by plating. Biofilms formed in the wells of a 96-well plate by *P. acnes* LMG 16711, LMG 16712 and LMG 16715 consisted of $10^6 - 3 \times 10^7$ CFUs per well (Fig. 1). For *P. acnes* LMG 16711 (the only isolate investigated) this corresponded to absolute fluorescence values of 11,000–13,000 per well when tested in the resazurin assay (data not shown).

3.2. *Resistance of* P. acnes *biofilm to antimicrobial agents*

We sought to assess: (i) whether *P. acnes* cells grown in a biofilm are more resistant to antimicrobial agents than planktonically grown cells; and (ii) whether currently used antimicrobial agents can disrupt existing *P. acnes* biofilms or kill the cells within these biofilms. These experiments were carried out using *P. acnes* LMG 16711.

Regarding their ability to inhibit proliferation of planktonic cells, it was shown that all components tested completely inhibited bacterial growth (data not shown). However, when the same compounds were added to the wells of microtiter plates in which 24 h-old biofilms had been allowed to develop, a different picture was obtained (Fig. 2). Based on results of crystal violet staining, seven agents (or combinations of agents) resulted in a significantly reduced biofilm biomass compared to the control: 0.5% erythromycin ($P < 0.001$), 2% salicylic acid ($P < 0.001$), 0.1% triclosan ($P < 0.001$), 0.5% minocycline ($P < 0.001$), 5% benzoyl peroxide +0.5% erythromycin ($P < 0.001$) and 5% benzoyl peroxide +1% clindamycin ($P < 0.01$). For 1% clindamycin we noted a trend towards significance ($P = 0.045$). However, 0.3% doxycycline, 0.5% oxytetracycline, 30 mM azelaic acid, 2.5% or 5% benzoyl peroxide did not result in a significantly decreased total biofilm biomass. In order to determine the fraction of surviving cells following treatment with antimicrobial agents for 24 h, we used a resazurin-based viability stain. As can be seen from Fig. 2, only 30 mM azelaic acid, 0.1% triclosan, and 5% benzoyl peroxide combined with either 0.5% erythromcyin or 1% clindamycin resulted in a meaningful degree of killing (i.e. >99.9% reduction). Antimicrobial products like 2% salicylic acid, 0.5% minocycline and 1% clindamycin resulted in less reduction (<99.0%), while the other products (0.5% erythromycin, 0.3% doxycycline, 0.5% oxytetracycline and 2.5 or 5% benzoyl peroxide) were only moderately bactericidal and did not result in biologically meaningful reductions (reductions <99.0%) of the number of viable cells. The bactericidal effects of high concentrations of clindamycin [35], azelaic acid [1] and triclosan [32] have previously been described. A remarkable effect of salicylate on *Staphylococcus epidermidis* biofilms, both in terms of prevention of biofilm formation and in terms of biofilm eradication, has also been reported. This antibiofilm effect is thought to be caused by a reduction in the production of matrix components, including polysaccharides [25]. Of all tetracyclines tested, minocycline appeared most active, both for removal of biofilm and for killing of sessile cells. Benzoyl peroxide alone had no effect on *P. acnes* biofilms, but combined with high concentrations of erythromycin or clindamycin (which have only moderate to low activity against sessile *P. acnes* cells by themselves), this strongly oxidizing agent becomes highly bactericidal. It is not unlikely that inhibition of protein synthesis by these antibiotics makes cells more susceptible to reactive oxygen species generated by benzoyl peroxide.

Our data confirm [6] that biofilm dispersal and removal do not necessarily correlate with biofilm killing: agents that kill sessile cells may fail to reduce the total biomass (e.g. 30 mM azelaic acid), and conversely, agents that result in reduction of the total biomass may leave a large fraction of living cells behind (e.g. 0.5% erythromycin).

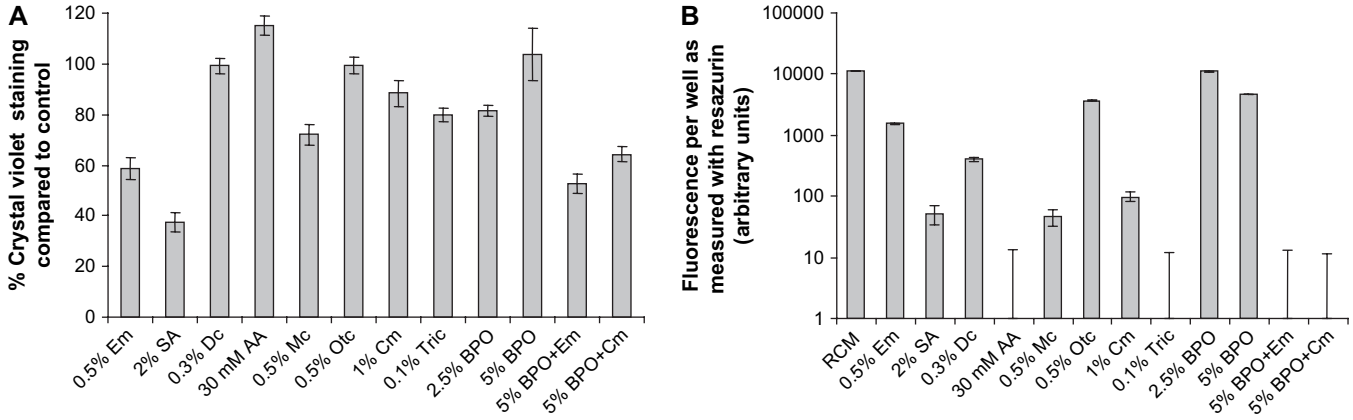

Fig. 2. A. Relative decrease in biofilm biomass of *P. acnes* LMG 16711 in the presence of various antimicrobial agents, as measured by crystal violet staining. Values are expressed relative to the positive control (no antimicrobial agent added) following 24 h of biofilm formation. B. Average absolute fluorescence values per well as measured by resazurin-based viability staining. Error bars indicate standard error of the mean. Abbreviations: RCM, reinforced clostridial medium; Em, erythromycin; SA, salicylic acid; Dc, doxycycline; AA, azelaic acid; Mc, minocycline; Otc, oxytetracycline; Cm, clindamycin; tric, triclosan; BPO, benzoyl peroxide.

3.3. Detection of putative virulence factors

Using 4-MU-based fluorogenic substrates, the lipase activity present in supernatants of planktonic and sessile *P. acnes* cultures was determined. As can be seen in Fig. 3, lipase activity was considerably higher in supernatants from sessile cells than in those from planktonic cells. The slope of the fluorescence-time curves of the three biofilm-forming *P. acnes* strains for the different substrates can be used as a quantitative measure of enzyme activity. These data (Table 1) clearly indicate drastically increased lipase activity in supernatants derived from biofilms. The overproduction of lipases is most obvious in acne isolates LMG 16711 and LMG 16712, with relative slopes ranging from 6216% (LMG 16711, oleate) to 1287% (LMG 16712, palmitate). No phospholipase, protease, DNase or hemolytic activity could be detected either in supernatant from planktonic or in that from sessile cells (data not shown). It is thought that *P. acnes* lipases play an important role in the pathogenesis of acne, as production of irritant fatty acids may contribute significantly to inflammation [18]. In addition, the lipase itself can act as a neutrophil attractant [22]. Previously it was also shown that free fatty acids increase the adhesion of *P. acnes* cells and promote colonization of the sebaceous follicle [15]. Our observation that lipase activity is higher in *P. acnes* biofilms and that this is especially the case for acne isolates supports the suggestion that *P. acnes* biofilm formation may be an important factor in the pathogenesis of acne. The fact that no other putative virulence factors previously demonstrated in *P. acnes* could be detected may reflect the culture-condition-dependent expression of these factors and/or strain-specific differences [17,26].

Table 1
Relative slope of fluorescence-time curves of the three *P. acnes* strains for the different 4-MU substrates

Substrate	LMG 16711	LMG 16712	LMG 16715
4-MU oleate	6216	2039	306
4-MU palmitate	1299	1287	779
4-MU stearate	2311	1647	1018

The relative slope is calculated as follows: (slope of curve for sessile cells/slope of curve for planktonic cells) × 100. Slopes of curves are based on results obtained in at least two independent experiments.

3.4. Detection of AI-2 in supernatant of P. acnes planktonic and sessile cells

Thus far, quorum sensing has not been described in *P. acnes*, but a closer look at the *P. acnes* genome sequence [2]

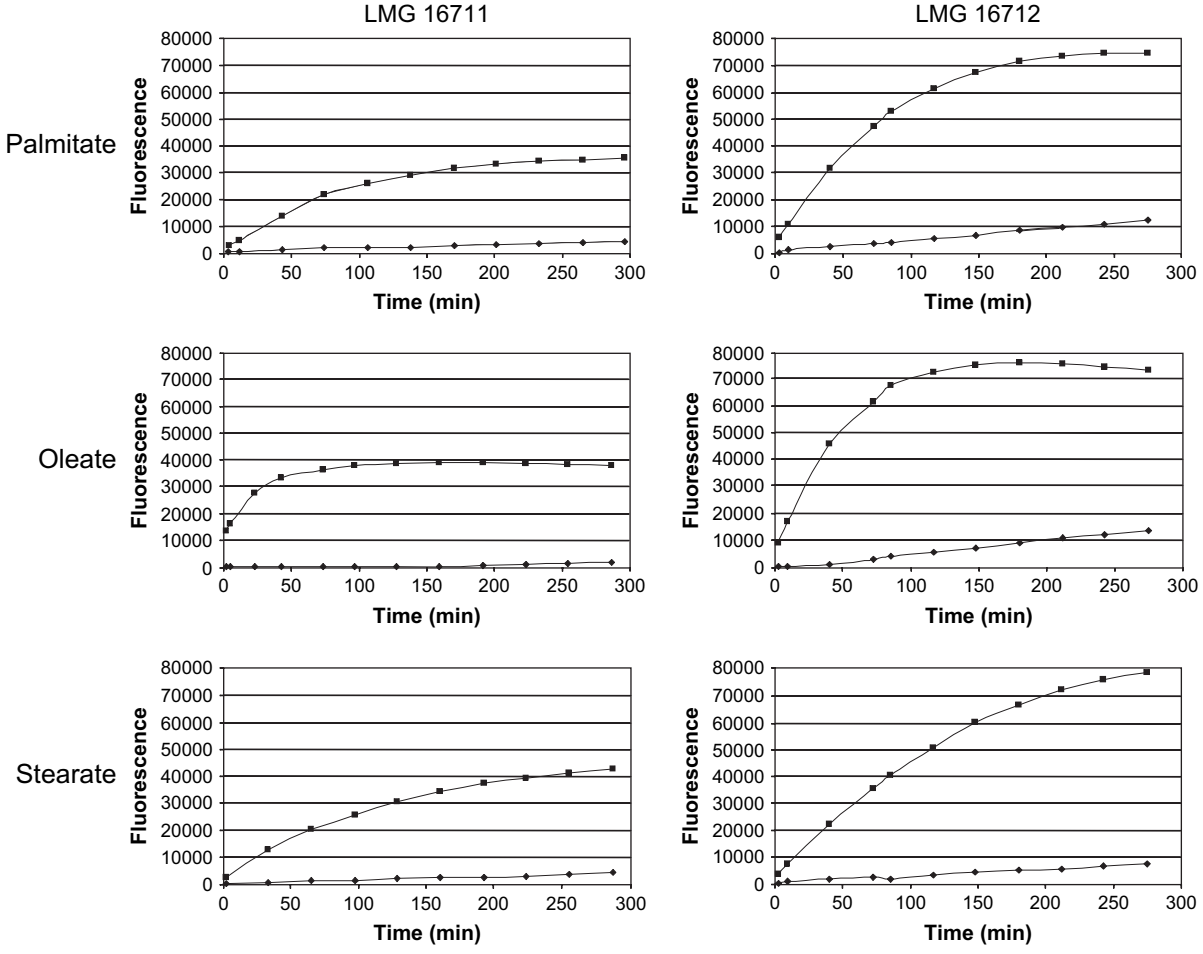

Fig. 3. Lipase activity of *P. acnes* strains LMG 16711 and LMG 16712, measured using 4-MU palmitate, 4-MU oleate and 4-MU stearate as substrates. Lipase activity of sessile cells is shown as squares.

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Propionibacterium acnes KPA171202 (PPA0450)    MREDGFMAHKMNVESFNLDHTKVAAPFVRVADVKHLPAGDTLTKYDVRFCQPNKEHLDMP 60
Staphylococcus aureus USA300 (SAUSA300_2088)   -------MTKMNVESFNLDHTKVVAPFIRLAGTMEGLNGDVIHKYDIRFKQPNKEHMDMP 53
Listeria monocytogenes F2365 (LMOf2365_1306)   ------MAEKMNVESFNLDHTKVKAPFVRLAGTKVGVHGDEIYKYDVRFKQPNKEHMEMP 54
Streptococcus mutans UA159 (SMU.474)           ------MTKEVTVESFELDHIAVKAPYVRLISEEFGPKGDLITNFDIRLVQPNEDSIPTA 54
Clostridium perfringens SM101 (CPR_0167)       ---------MVKVESFELDHTKVKAPYVRKAGIKIGPKGDIVSKFDLRFVQPNKELLSDK 51
Vibrio harveyi (AF120098)                      ---------MPLLDSFTVDHTRMNAPAVRVAKTMQTPKGDTITVFDLRFTAPNKDILSEK 51

Propionibacterium acnes KPA171202 (PPA0450)    AVHSLEHSFAECVRNHSD----AVIDFGPMGCQTGFYLIMIGEPDVSGTCELVETTLRDI 116
Staphylococcus aureus USA300 (SAUSA300_2088)   GLHSLEHLMAENIRNHSD----KVVDLSPMGCQTGFYVSFINHDNYDDVLNIVEATLNDV 109
Listeria monocytogenes F2365 (LMOf2365_1306)   ALHSLEHLMAELARNHTD----KLVDISPMGCQTGFYVSFINHSDYDDALEIIATTLTDV 110
Streptococcus mutans UA159 (SMU.474)           GLHTIEHLLAKLIRQRID----GMIDCSPFGCRTGFHLIMWGKHTTTQIATVIKASLEEI 110
Clostridium perfringens SM101 (CPR_0167)       GMHTLEHFLAGFMREKLD----DVIDISPMGCKTGFYLTSFGDIDVKDIIEALEYSLSKV 107
Vibrio harveyi (AF120098)                      GIHTLEHLYAGFMRNHLNGDSVEIIDISPMGCRTGFYMSLIGTPSEQQVADAWIAAMEDV 111

Propionibacterium acnes KPA171202 (PPA0450)    LKL---NTTPAANEVQCGWGANHSIKAAQEAAHTMLNHRDEWEQ---------VMA----- 160
Staphylococcus aureus USA300 (SAUSA300_2088    LNA---TEVPACNEVQCGWAASHSLEGAKTIAQAFLDKRNEWHD---------VFGTGK-- 156
Listeria monocytogenes F2365 (LMOf2365_1306)   LAA---TEVPACNEVQCGWAASHSLEGAKALAAEFLDKRDEWKN---------VFGE---- 155
Streptococcus mutans UA159 (SMU.474)           ANTISWKDVPGTTIESCGNYKDHSLFSAKEWAKLILKQGISDDP---------FERHLV-- 160
Clostridium perfringens SM101 (CPR_0167)       LEQ---EEIPAANELQCGSAKLHSLELAKSHAKQVLEN-GISDK---------FYVE---- 151
Vibrio harveyi (AF120098)                      LKVENQNKIPELNEYQCGTAAMHSLDEAKQIAKNILEVGVAVNKNDELALPESMLRELRID 172
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Fig. 4. Alignment of the LuxS protein sequence from *P. acnes* with homologs of related organisms and of *V. harveyi*. Shaded residues are conserved in all sequences. *P. acnes* specific residues are highlighted in bold.

revealed that it contains a gene (PPA0450) encoding a *V. harveyi* LuxS homolog (Fig. 4). However, no other genes involved in the signal transduction pathway in *V. harveyi* appear to be present. This is in agreement with previous data showing that the LuxS enzyme is widespread in bacteria, but that other proteins have much more restricted distribution, suggesting that *P. acnes* may use alternative proteins in the AI-2 signalling cascade [38]. Using the *V. harveyi* biosensor, we could demonstrate the production of AI-2, both in planktonic and sessile *P. acnes* LMG 16711 cells (Fig. 5). Production was approximately 1.5 times higher in young (4 h) biofilms compared to planktonic cells, but more than 3 times higher in mature biofilms. Although data on the role of AI-2 in bacterial virulence are often contradictory (for an overview see ref. [38]), there is a general consensus that the presence of AI-2 can have significant effects on expression of virulence factors (e.g. in *Vibrio cholerae* [46]), indicating a possible role for increased AI-2 production by *P. acnes* biofilms in the pathogenesis of acne.

3.5. Concluding remarks

Although evidence for the involvement of *P. acnes* biofilms in the pathogenesis of acne vulgaris remains circumstantial, results of the present study indicate that multiple *P. acnes* strains, including acne isolates, can form biofilms in vitro. Our data also show that sessile *P. acnes* cells are more resistant to antimicrobial agents than planktonic cells. This could help explain the frequent failure of antimicrobial therapy in the treatment of acne. As a consequence of this, it seems appropriate to test the efficacy of new antimicrobial agents or combinations of agents not only on planktonic *P. acnes* cells but also on sessile cells. We also noted drastically increased lipase activity in supernatant from sessile cells compared to planktonic cells. As lipase activity may contribute to irritation and inflammation, the formation of *P. acnes* biofilms in vivo may be an important trigger for the inflammation often seen in acne. Our experiments also demonstrated higher levels of AI-2 in biofilms. The exact role of this increased AI-2 production in *P. acnes* biofilms is not clear, but it may very well contribute to an increase in the expression of particular virulence genes, again contributing to the pathogenesis of acne. Further in vitro and in vivo studies with additional acne-derived *P. acnes* isolates will be required to determine whether *P. acnes* forms biofilms in human pilosebaceous follicles.

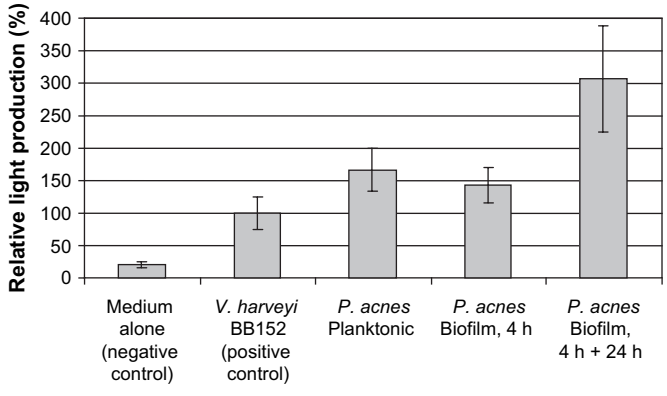

Fig. 5. Relative light production by a *V. harveyi* sensor strain induced by supernatant of planktonic and sessile *P. acnes* LMG 16711 cells. Error bars indicate standard error of the mean.

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