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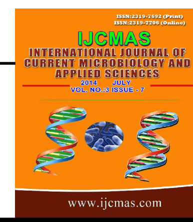

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Original Research Article

Pseudomonas fluorescens, a forgotten member of the human cutaneous microflora sensible to skin communication and defense peptides

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Keywords

Inter-Kingdom Communication; Skin; Pseudomonas fluorescens; Substance P; Cathelicidins; Defensin; Cytotoxicity; Adhesion; Biofilm

A B S T R A C T

Pseudomonas fluorescens has been ignored for a long time as a member of the cutaneous microflora. In the present study we investigated the effect of Substance P (SP), the major skin neuropeptide, and skin antimicrobial peptides, the cathelicidin LL37 and the defensin HBD2, on the human skin strain *P. fluorescens* MFP05. SP (10-6M) was without effect on the growth and virulence of MFP05 but increased the adhesion and invasive potential of the bacterium on the human keratinocyte cell line HaCaT (+41 ± 3 and +33 ± 2 %, respectively). The biofilm formation activity of MFP05 was also affected by SP suggesting an effect of the peptide on the expression of bacterial adhesin(s). *P. fluorescens* MFP05 was poorly sensible to antimicrobial peptides. At sub-lethal concentration (1 µg/mL) HBD2 induced a marked decrease of the bacterium cytotoxicity (-52.4 ± 4.5 %) whereas LL37 was without effect. The two peptides also increased the biofilm formation activity of MFP05 but their effects were not additives and LL37 had a transient effect on the biofilm structure. This study reveals that *P.fluorescens* is sensible to naturally occurring human skin peptides and can develop adaptations aimed at improving its skin tolerance

Introduction

Pseudomonas fluorescens in one of the more abundant environmental Gram negative-bacterium and, as an interface with the environment, skin is contaminated and colonized by these microorganisms. Metagenomic studies indicate that in skin areas such as the inner elbow, Pseudomonas, and particularly bacteria of

the fluorescent group should represent the principal bacterial community (Grice et al., 2008). However, little is known about these skin associated P. fluorescens since in contrast they are very rarely detected in cultured based studies (Gao et al., 2007; Costello et al., 2009) although they were found responsible of contamination during blood collection for transfusions (Murray et al., 1987; Gibb et al., 1992) and are then potentially viable. In fact, it is unclear if these germs are real metabolically active skin associated bacteria or if DNA sequences identified in metagenomic studies only result from transient contaminations by microorganisms from air, soil or water. Otherwise, as the skin microbial community is highly adapted to this environment, it is possible that skin *P.fluorescens* developed auxotrophy to unknown eukaryotic factors. Indeed, although Pseudomonas are known for their huge adaptation potential, it has been shown that fluorescent Pseudomonas can be dependent of specific metabolites (Marek-Kozaczuk et al., 2005) or amino acids (Sahu and Ray 2007). Alternatively or in complement, the physiology of these bacteria should be also modulated by local skin factors since, as now widely recognized, fluorescent Pseudomonas can detect different eukaryote communication molecules (Lesouhaitier et al., 2009; Dagorn et al., 2013a).

In a recent study, we collected on the skin of a healthy volunteer a *P.fluorescens* strain, designated as MFP05, that was characterized by biochemical tests, 16S rRNAsequencing and mass spectrometry whole proteome analysis (Hillion et al., 2013). This bacterium is growing on LB medium but, as some clinical variants of the species (Chapalain et al., 2008), it can multiply at 37°C and then does not behave as a typical psychrotropic P.fluorescens. We have also demonstrated that human skin associated Gram positive bacteria, such as Bacillus cereus, Staphylococcus aureus and Staphylococcus epidermidis can detect Substance P (SP), the major skin neuropeptide, and respond by a rapid and massive increase of virulence (Mijouin et al., 2013). SP is also a weak antimicrobial peptide (Hansen et al., 2006) and in skin this molecule could act by other mechanisms since it also stimulates the release of antimicrobial peptides of the cathelicidins (LL37) and defensins (HBD2) families (Brogden, 2005). SP is present in sweat and its concentration increases with stress and nervous disorders reaching micromolar values at the vicinity of nerve endings (Cizza et al., 2008). Conversely, in healthy skin, the concentration of LL37 and HBD2 is low, about 2 µg/mL (Bals et al., 1999; Schaller-Bals et al., 2002; Lai and Gallo, 2008). Then, skin bacteria are potentially exposed to SP and/or endogenous antimicrobial peptides at sublethal concentrations. These environmental conditions can have major consequences on bacterial physiology since it has been shown that, at low concentrations, antibacterial peptides can modulate the virulence of bacteria without acting on their growth rate (Hancock and Scott, 2000). Moreover, understanding and controlling theses relations between cutaneous bacteria and skin peptides should be essential for the safety of cosmetics (Orth, 2010) and medical devices (Chopra et al., 2012).

In this study we investigated the effect SP, LL37 and HBD2 on the growth, virulence and biofilm formation activity of the human skin strain of *P.fluorescens* MFP05.

Materials and Methods

Bacterial strains and culture conditions

Pseudomonas fluorescens MFP05 has been collected by swabbing on normal human skin.

This strain has been identified by phenotypic, metabolic, MALDI-Biotyper proteomic and 16S ribosomal RNA gene sequencing techniques (Hillion et al., 2013). For confocal microscopic studies, MFP05 was transformed by insertion of the pSMC2.1 plasmid, encoding for GFP and for kanamycin resistance (Bazire et al., 2007). This strain was designated as *P.fluorescens* MFP51. Bacteria were grown at 28°C in Luria-Bertani (LB) broth under 180 rpm agitation. For pre-treatment, bacteria were diluted at a ratio of 1:40 in fresh broth and the peptides were added at the beginning of the log growth phase. Before assays, bacteria were harvested by centrifugation (7000xg, 5 min) and washed three times with sterile physiological water to remove any trace of free peptide. Bacteria were then adjusted at the appropriate cell density. The density of the bacterial suspension was determined by measuring OD580 using a ThermoSpectronics spectrophotometer (Cambridge, UK). The bacterial density and the absence of contamination were controlled by plating. Transformed strains were grown in the same conditions except that kanamycin (100 µg/mL) was added. The viability of bacteria in the eukaryotic medium and under the different culture conditions and times was controlled in preliminary studies (data not shown).

Chemicals

Substance P (SP) and the reversed sequence peptide (SPrev) were obtained from Polypeptides (Strasbourg, France). Antimicrobial peptides (LL-37 and HBD2) were purchased from Innovagen (Lund, Sweden).

Cytotoxicity assays

The cytotoxic activity of bacteria was studied on the human keratinocyte cell line HaCaT. HaCaT cells, provided by Cell Lines Services (CLS, Eppelheim, Germany) were grown at 37°C under 5% CO2 in Dulbecco's modified Eagle's medium (DMEM, Lonza) containing 25 mM glucose supplemented with 10% inactivated fetal bovine serum, 2mM L-glutamine (Lonza) and antibiotics (penicillin 100 IU/mL and streptomycin 100 µg/mL). Cells were used between passages 41 and 65. Cells were seeded in 24-well plates at a final density of 5.105 cells per well, and grown for at least 48h. One day before infection assay, fresh serum-free medium without antibiotics was added. The cytotoxic potential of the bacteria was determined by measurement of lactate dehydrogenase (LDH) release by HaCaT cells upon cytoplasmic membrane destabilization. LDH is a stable cytosolic enzyme whose release is considered as an indicator of necrosis. HaCaT cells at 90% confluence were infected with 1 mL of mid-log phase bacterial suspension at a bacterium-to-cell ratio of 10:1. The amount of LDH released by HaCaT cells was determined after 15 h of incubation using the Cytotox 96 enzymatic assay (Promega, Charbonnières, France), as previously described (Picot et al., 2003). Control studies performed using bacteria alone showed that note of the strains used in the present study produced metabolites interfering with the assay.

Binding and invasion assays

HaCaT cells were infected for 1 h with *P.fluorescens* MFP05. A volume of 300 µL

of mid-log phase bacterial suspension resuspended in DMEM without fetal calf serum was added, at a bacterium-to-cell ratio of 10:1. In a first series of experiments, after bacteria-cell contact, HaCaT cells were gently washed six times with DMEM to remove non adherent bacteria and then disrupted with 1 mL 0.9% Triton X100. The total number of cell associated bacteria (intra- and extra-cellular) was then counted by plating serial dilutions on Tryptone Soya Agar medium (TSA). Invasive bacteria were quantified by the gentamicin protection assay as described by Mezghani-Abdelmoula et al. (2004). In these studies, after infection with bacteria and before washing, HaCaT cells were incubated with 100 µg/mL gentamicin to kill extracellular bacteria. HaCaT cells were then washed and lysed by as previously described and the number of invading bacteria released from the cells was counted. Cells surface adherent bacteria were calculated by subtraction of the number of invasive bacteria to the total number of cell associated bacteria. For each assay, serial dilutions of the whole bacterial inoculum were plated. The absence of effect of HaCaT cells lysis buffer on the viability of *P.fluorescens* MFP05 was controlled in a preliminary test.

Biofilm formation studies

The effect of the peptides on the biofilm formation activity of P. fluorescens MFP05 was investigated by the crystal violet technique adapted from O'Toole and Kolter (1998) and by confocal laser scanning microscopy. In the crystal violet technique, an aliquot of 100 µL of a bacterial culture was adjusted to an OD580 = 0.4 was layered in each well of a polystyrene microtitration plate. Bacteria were exposed to the peptides since the beginning of culture and during the whole biofilm formation period. After 24h of incubation at 37°C the bacterial suspension was removed. Bacteria and matrix bound to the wells were carefully rinsed and stained with crystal violet (0.1%) for 30 min. After rinsing again 3 times with distilled water, the dye was recovered by adding 100 µ L SDS (1% in sterile water) and the OD595 was measured.

Confocal microscopy studies were realized using the GFP transformed strain *P.fluorescens* MFP51. Bacteria were inoculated at an OD580=0.08 into fresh LB medium and grown for 15 h in the absence or presence of the peptides. Bacteria in late stationary growth phase were then washed twice by centrifugation at 7000xg for 10 minutes and resuspended in 25mL sterile physiological water. The suspension was adjusted to an OD580=1, poured in a 140mm Petri dish containing 3 sterile glass slides, and incubated for 2h. The glass slides were then washed twice with sterile physiological water to remove non-adherent bacteria. One slide was immediately heath fixed to observe the initial adhesion. The two other slides were then recovered by fresh LB medium containing or not the same tested peptide and incubated for 5 or 24 h without agitation. These slides were washed and fixed as previously described, and immediately observed using a LSM 710 inverted confocal laser-scanning microscope (Zeiss).

Three-dimensional (3D) images, ortho cuts and biofilm thickness values were generated using Zen® 2009 software.

Statistical analysis

All results are expressed as means ± standard error (SEM) calculated over a minimum of three independent experiments. Statistical differences were estimated using

the Student's t test and were noted as*, ** and *** for p-values < 0.05, <0.01 and <0.001, respectively. In confocal microscopy studies, the thickness of the biofilms was calculated from a minimum of 20 measures in different fields.

Results and Discussion

Effect of SP on the cytotoxicity, cell binding potential and invasivity of *P.fluorescens* on keratinocytes

The effect of *P.fluorescens* on keratinocytes was studied using the human skin strain MFP05 and the human keratinocyte cell line HaCaT. Preliminary controls showed that exposure of *P.fluorescens* MFP05 to SP (10-6 M) during the whole growth phase or at the beginning of the stationary phase did not affect the growth kinetics of the bacterium. The peptide was not tested at higher concentrations as SP has non-specific antibacterial activities at non physiological doses > 10-5 M (Hansen et al., 2006). As revealed by the LDH release assay, treatment of *P.fluorescens* with SP (10-6 M) did not modify its cytotoxic potential on HaCaT keratinocytes, (Fig. 1A). SPrev, which was used as control of SP, was also without effect on *P.fluorescens* MFP05 cytotoxicity. Conversely, as shown in Figure 1B, SP (10-6M) induced a marked increase of the adhesion of P. fluorescens on keratinocytes (+41 ± 3 %; p<0.001). This effect was accompanied of a raise of the invasive behavior of the bacteria (+33 ± 2 %; p<0.001). As observed in cytotoxicity studies, SPrev was characterized by a total absence of effect on the binding and invasive potentials of *P.fluorescens* MFP05.

Effect of SP on the biofilm formation activity of *P.fluorescens*

The effect of SP on the biofilm formation activity of *P.fluorescens* was studied by confocal microscopy using the GFP-transformed strain MFP51. The absence of effect of the transformation on the sensitivity of the bacterium to SP was controlled in preliminary growth kinetics, cytotoxicity, cell binding and invasivity studies. As shown in Figure 2A the initial adhesion of the bacterium after 2 hours was not modified by SP. However, after 5 hours we observed that the biofilm formed by SP treated bacteria was characterized by the presence of dense bacterial clusters mixed with dispersed bacteria whereas on control slides the biofilm was formed of well structured rows separated by water channels. The difference of morphology of the biofilms formed by control (or SPrev treated) and SP treated bacteria was more visible after 24h. Whereas control bacteria formed a continuous biofilm, the biofilm of SP treated bacteria appeared disorganized with dispersed aggregates. Although the surface coverage was completely different, the mean thickness of the biofilms of control and SP treated bacteria was the same (Fig. 2B).

Effect of antimicrobial peptides on the growth kinetics of P. fluorescens

The effect of LL37 and HBD2 on the growth kinetics of *P.fluorescens* MFP05 was studied at concentrations ranging from 1 to 100 µg/mL. P. fluorescens MFP05 was poorly sensible to LL37 (Fig. 3A). The generation time of the bacterium (slope of the exponential growth phase) was not modified. At concentrations 1 and 5 µ g/mL the effect of LL37 on the growth kinetics of MFP05 was null or marginal and even at 50 µg/mL we only observed a limited reduction (-12%) of the stationary phase plateau. At the highest dose studied (100 µg/mL), LL37 appeared to accelerate the decline

phase of the bacterium which occurred after 12h of culture.

The effect of HDB2 on the growth of P. fluorescens MFP05 was more important (Fig. 3B). The generation time was not modified but a limited increase of the lag-time was observed for concentrations of HBD2 > 25 µg/mL. In addition, a dose related decrease of the stationary phase level was noted for concentrations ranging from 25 to 100 µg/mL. As we expected to investigate the effect of LL37 and HBD2 in sub-lethal conditions, we selected the concentrations 1 and 5 µg/mL for further studies.

Effect of sub-lethal concentrations of antimicrobial peptides on the cytotoxicity of P.fluorescens

The effect of LL37 and HBD2 on *P.fluorescens* MFP05 cytotoxicity was studied at a concentration of 1µ g/mL. Bacteria exposed to LL37 showed a limited, but non significant, increase of cytotoxicity (Fig. 4). Conversely, although HBD2 1µ g/mL did not modify the growth kinetic of the bacterium, a marked decrease of cytotoxicity (-52.4 ± 4.5 %, $p<0.001$) was noted. When the two peptides were associated at the same concentration of 1µg/mL, there was no additivity or potentiation of their effects and the reduction of P. fluorescens cytotoxicity was of the same as observed with HBD2 alone (-49.4 ± 8.3 %, $p<0.01$).

Effect of sub-lethal concentrations of antimicrobial peptides on the biofilm formation activity of P. fluorescens

In a first step, the effect of LL37 and HBD2 on the biofilm formation activity of *P.fluorescens* MFP05 was studied using the crystal violet technique. As shown in Figure 5A, LL37 at concentrations 1 and 5 µg/mL induced a significant increase of biofilm production (+31 ± 14%, $p<0.05$ and +50 ± 2%, $p<0.001$, respectively). HBD2 was also leading to an increase of biofilm formation but this effect was not significant, essentially because of the high dispersion of the values. The effects of LL37 and HBD2 were not additive since the association of the two molecules at a concentration of 1 µg/mL was leading to an increase of biofilm of the same range as observed using LL37 1 µg/mL alone (+41 ± 4%, $p<0.01$).

In complement, the activity of LL37 on the biofilm structure was studied by confocal microscopy using the GFP transformed strain MFP51. The initial adhesion of the bacteria was not modified by LL37 and the biofilm formed after 2 h incubation was identical to that obtained in the absence of treatment. Differences between the biofilms formed by control and LL37 treated bacteria appeared after 5h incubation (Fig. 5B). As previously noted, control biofilms were presenting distinct bacterial rows, whereas the biofilms formed by LL37 treated bacteria were dense and homogeneous. However, this effect of LL37 was transient since after 24 h incubation the structure of the biofilms made by control and LL37 treated bacteria was identical.

Because of its large genome and richness in regulatory genes, P. fluorescens is a highly adaptable bacterium (Silby et al., 2011) and since it is present in abundance in the environment, it is logical that it was found on the skin and adapted to this specific niche. However, skin is not a friendly environment for bacteria, and particularly for P. fluorescens.

Fig.1 Effect of Substance P (SP) 10-6 M on the cytotoxic activity of *Pseudomonas fluorescens* MFP05 (A) and on its binding and invasive potential (B). Control studies were realized using the reversed sequence peptide (SPrev) (★★★= $p < 0.001$).

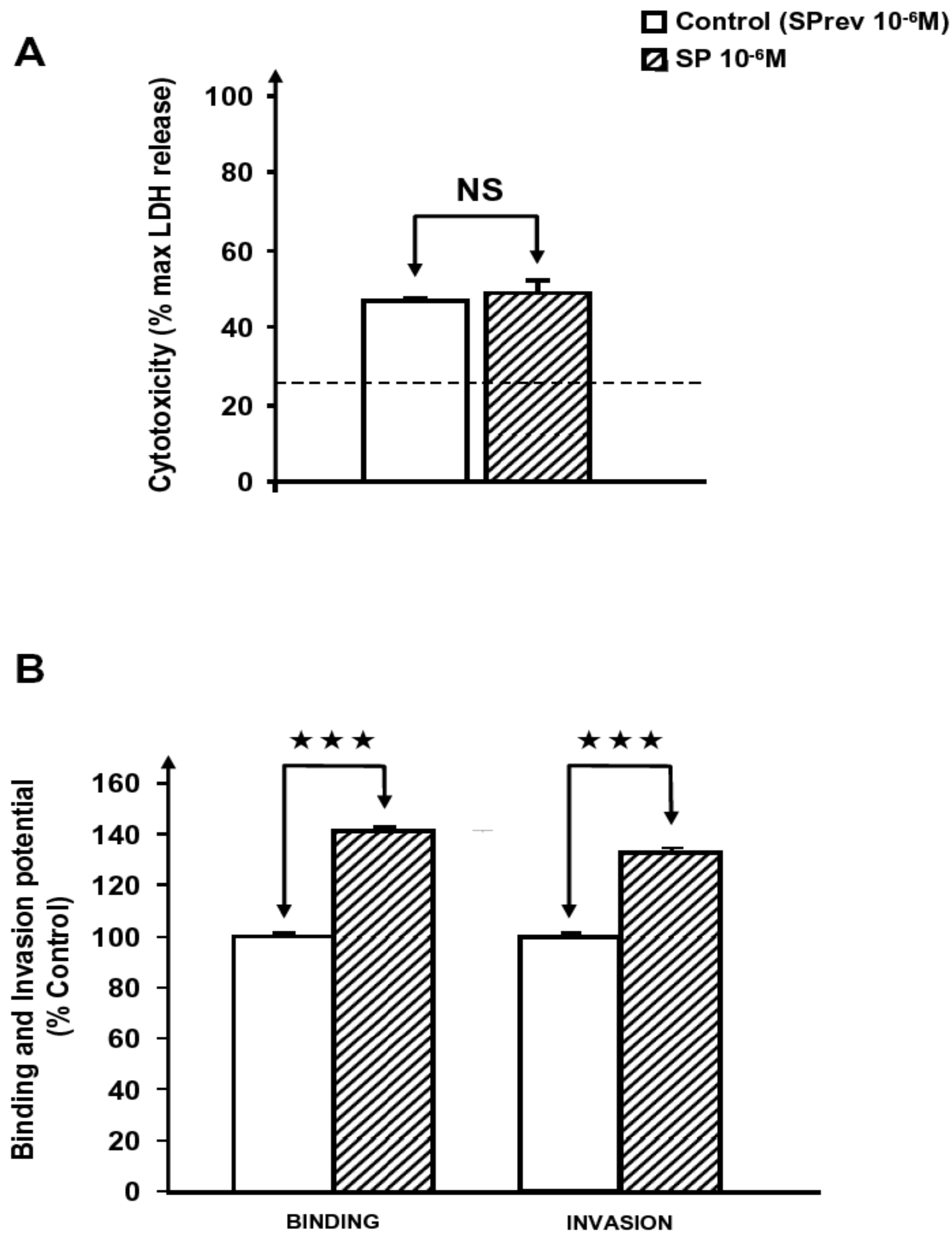
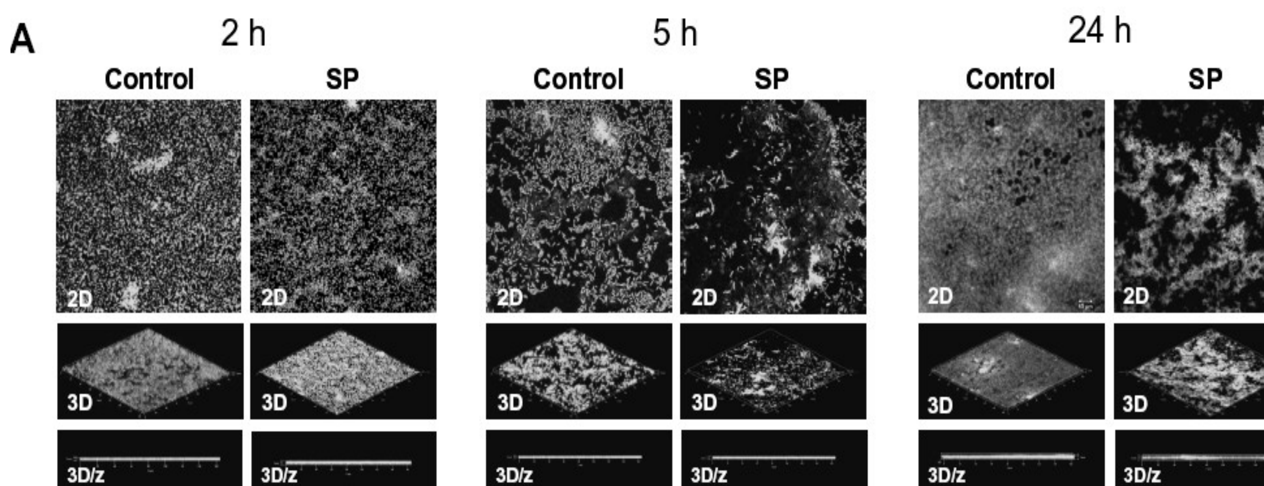


Fig.2 Effect of Substance P (SP) 10-6 M on the biofilm formation activity of *Pseudomonas fluorescens* MFP05. The biofilm formation was observed after 2, 5 and 24 h. Two dimensions (2D) and reconstructed three-dimensions (3D) and ortho cuts (3D/z) images showed that the structure of the biofilm was modified (A). In contrast, the mean thickness of the biofilm was unchanged after 2, 5 and 24 h incubation with SP (B). Control studies were realized using the reversed sequence peptide (SPrev) (NS = non significant).



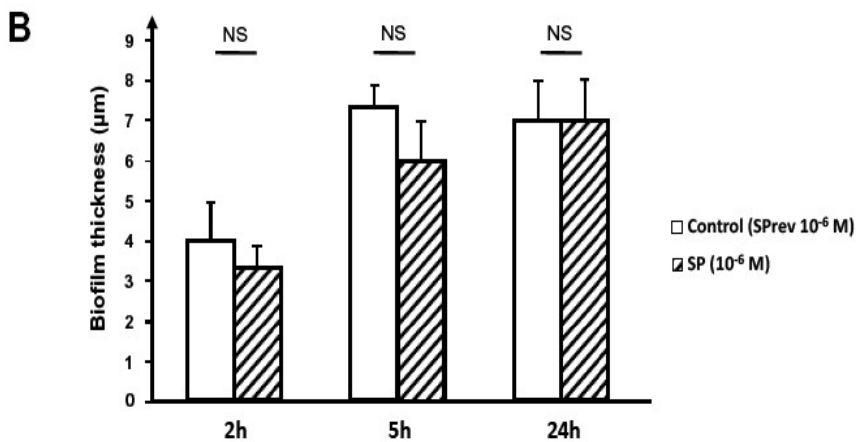


Fig.3 Effect of the cathelicidin (LL37) (A) and defensin (HBD2) (B) on the growth kinetics of *Pseudomonas fluorescens* MFP05.

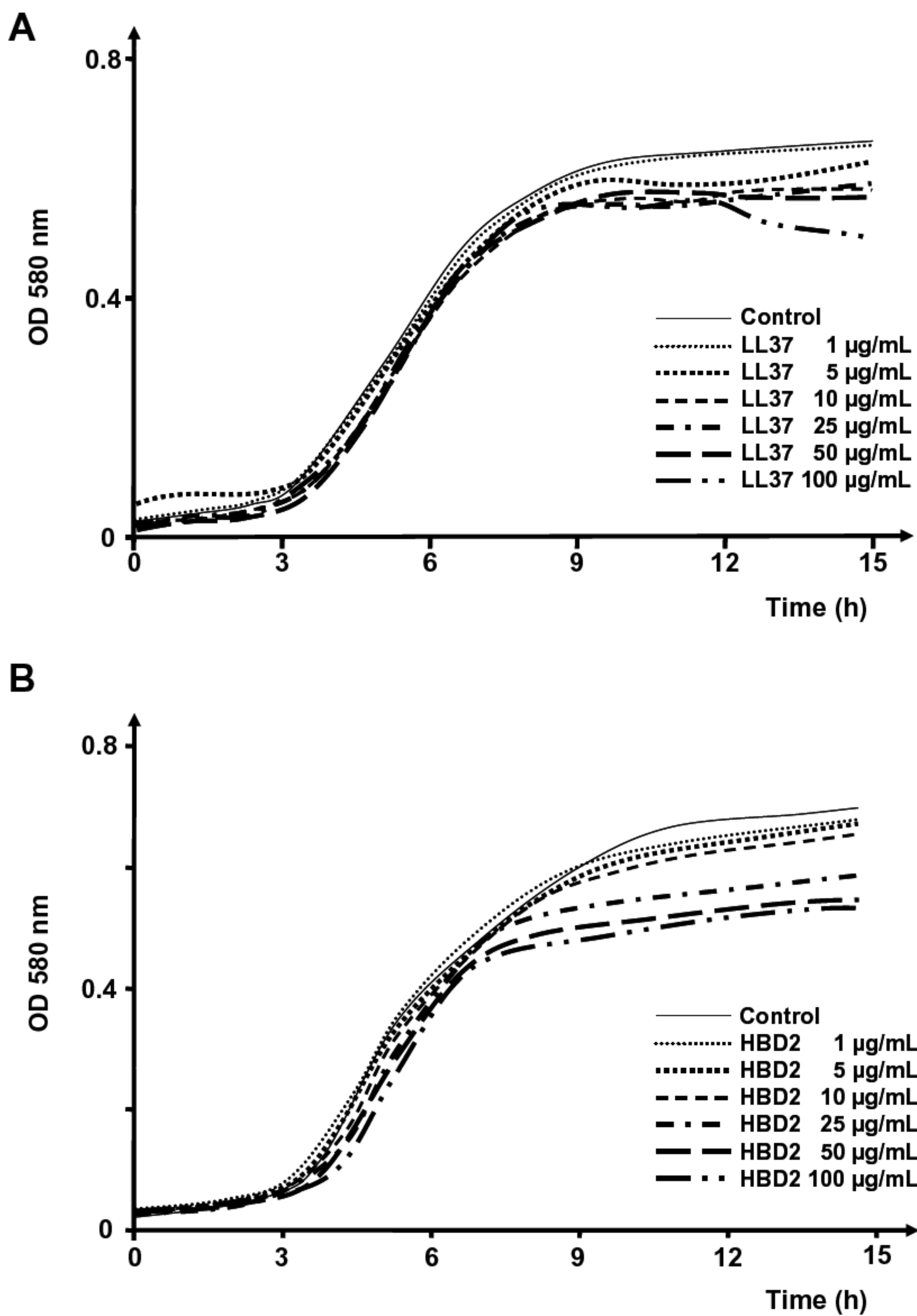


Fig.4 Effect of the cathelicidin (LL37) and defensin (HBD2) alone (1 µ g/mL) or in association (1 µg/mL each) on the cytotoxic activity of *Pseudomonas fluorescens* MFP05. (★★= $p < 0.01$; ★★★= $p < 0.001$).

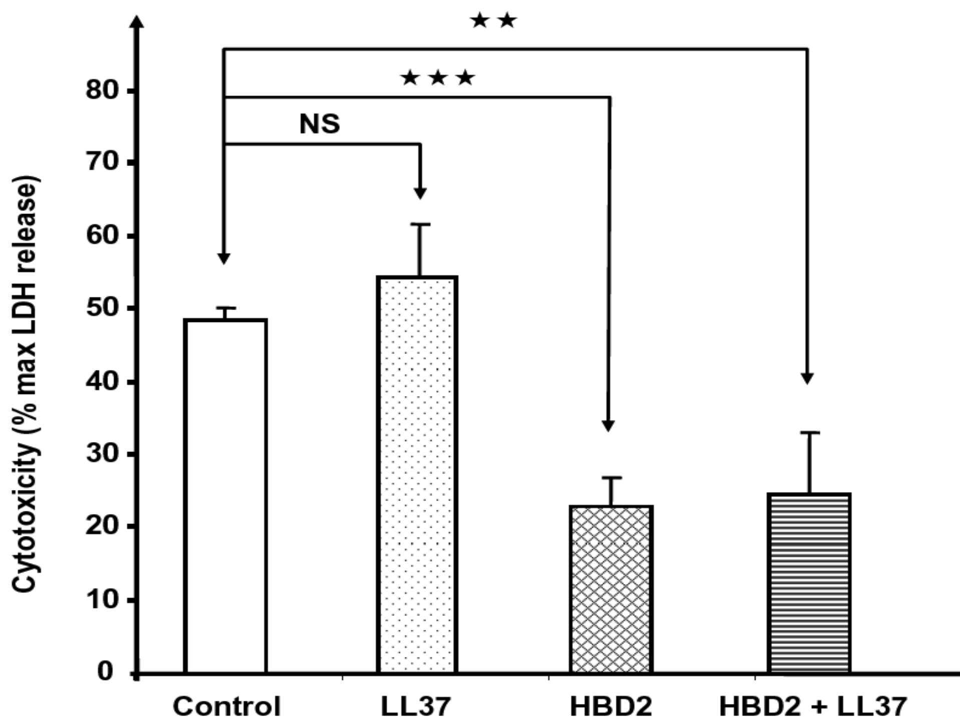
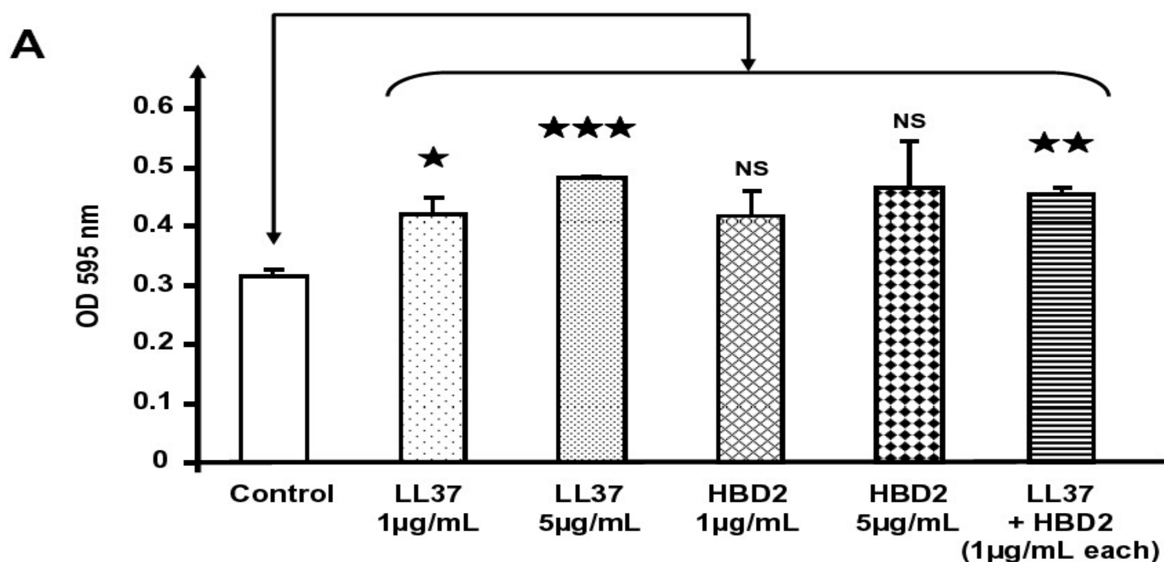
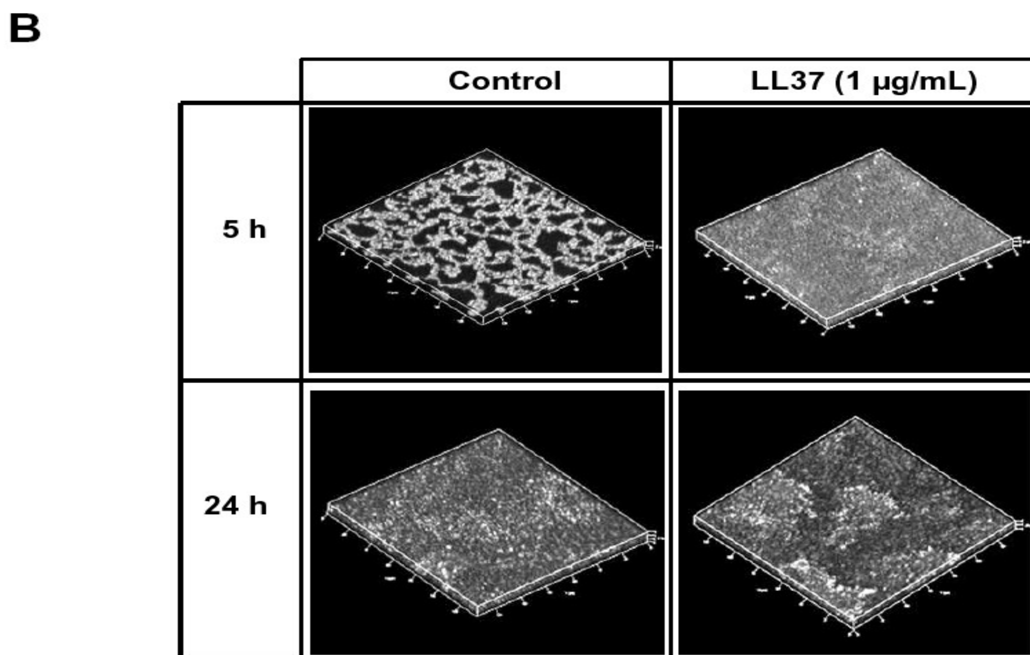


Fig.5 Effect of the cathelicidin (LL37) and defensin (HBD2) alone (1 or 5 µg/mL) or in association (1 µg/mL each) on the biofilm formation activity of Pseudomonas fluorescens MFP05. The biofilm formation was studied over 24 h by the crystal violet technique (A) and by confocal microscopy at 5 and 24 h (B). LL37 alone or in association with HBD2 induced a significant increase of biofilm formation. The structure of the biofilm was markedly modified after 5h of incubation but this effect was not observed after 24h. (NS = non significant; ★ = $p < 0.05$; ★★ = $p < 0.01$; ★★★ = $p < 0.001$).





A first reason is that the mean human skin temperature is about 33°C (Kopp and Haraldson, 1983) whereas *P.fluorescens* are generally considered as psychrotrophic organisms unable to grow above 32°C. In fact, P. fluorescens MFP05 was found able to grow at 28 and 37°C (Hillion et al., 2013) and in most of clinical strains of *P.fluorescens* previously identified this adaptation is leading to a higher basal cytotoxicity (Chapalain et al., 2008). This is in agreement with previous observations showing that in *P.fluorescens* the growth temperature is modulating its lipopolysaccharide (LPS) structure and endotoxic activity (Picot et al., 2004).

Skin surface is also generally dehydrated and rich in salts resulting from sweat evaporation (Menon et al., 2012). For hydrophilic bacterial species such as *P.fluorescens* this environment should provoke an osmotic stress to which these bacteria are known to respond by modifications of mobility and antibiotic susceptibility associated to differential outer membrane proteins expression (Guyard-Nicodème et al., 2008). We have also recently shown that Gram positive skin bacteria, are sensible to minimal variations (<10-6 M) of the principal skin neuropeptide (SP) (Mijouin et al., 2013). Taken together, these data show that on skin *P.fluorescens* is submitted to a high adaptation pressure and should have developed original behavior in response to this local environment.

Preliminary studies revealed that SP was without effect on the growth of *P.fluorescens* MFP05. That was also the case of other cutaneous bacteria such as B. cereus, S. aureus and S. epidermidis (Mijouin et al., 2013). Looking at the basal cytotoxicity of this bacterium, we noted that it was between that of two previously identified clinical strains MFY162 and MFN1032 (Chapalain et al., 2008). As all bacteria studied until now respond to SP by a rapid and massive increase of virulence (Mijouin et al., 2013), we were surprised to observe that the cytotoxicity of *P.fluorescens* MFP05 remained unchanged after exposure to SP. This result is not due to a lack of sensitivity to the peptide since in contrast SP-treated P. fluorescens MFP05 showed a significant increase of adhesion and invasivity in HaCaT keratinocytes. We have previously demonstrated that P. fluorescens can behave as an invasive microorganism (Mezghani-Abdelmoula et al., 2004) but it is the first time that we observe that this mechanism can be regulated by a local host factor. Moreover, as bacteria were rinsed to remove any trace of free peptide and then were the unique partner exposed to SP, this is indicating that P. fluorescens is not penetrating passively in HaCaT cells but is playing an active role in this process. Since invasivity and cell surface adhesion evolved in parallel and in the same range under the effect of SP, it is tempting to consider that the increase of invasivity is a consequence of a higher adhesion of the bacteria on HaCaT cells. This hypothesis is supported by the results of biofilm formation studies. Indeed, we saw structural differences of the biofilms but no variation of mean thickness suggesting that SP was modulating the expression of bacterial adhesion molecules but was not acting on the sessile to planktonic transition of lifestyle. This is also in agreement with cytotoxicity studies, since this event is normally associated to variations of virulence expression (Balasubramanian et al., 2013). As the cytotoxicity of *P.fluorescens* was

unchanged, this is indicating that SP was not affecting its LPS structure but had probably more subtle effects on adhesin(s), such as porins (Rebière-Huët et al., 2002) or Lap proteins (Newell et al., 2009). However, as the effects of SP were limited these changes should be very difficult to demonstrate by proteomic analysis.

Concerning the mechanism of action of SP on P. fluorescens, the total absence of effect of the reversed sequence peptide, as also observed in Bacillus and Staphylococci (Mijouin et al., 2013), suggests the existence of a common sensor system. The SP sensor in B. cereus was identified as the thermo unstable ribosomal elongation factor (Ef-Tu), a protein that should migrate on the bacterial membrane in stress conditions and acquire new ubiquitous binding or sensor activities (Mijouin et al., 2013). Pseudomonas also express an Ef-Tu analogue and it is interesting to note that in Pseudomonas aeruginosa, this protein was characterized as the binding site for gamma-aminobutyric acid, a major inter-kingdom communication molecule (Dagorn et al., 2013b). Then, the present results support the hypothesis of the existence of common pleiotropic bacterial sensors in Gram-positive and Gram-negative microorganisms.

As demonstrated in clinical and psychrotrophic strains (Madi et al., 2013) the growth kinetics of P. fluorescens is weakly affected by antimicrobial peptides such as LL37 and HBD2. The present results are in agreement with these observations, the growth kinetics of MFP05 being only marginally modified by the highest concentrations of LL37 and HBD2. In terms of cytotoxicity and biofilm formation, the responses of *P.fluorescens* MFP05 to LL37 and HBD2 were very different. LL37 was without effect on the cytotoxicity of MFP05 whereas HDB2 treated bacteria showed a marked reduced lethality on HaCaT cells. This is the opposite of what was observed on Caco2/TC7 enterocytes using the psychrotrophic strain *P.fluorescens* MF37, a spontaneous mutant of a milk strain (Madi et al., 2013). In this case, a preliminary treatment of the bacteria with HDB2 was associated to a marked increase of cytotoxicity. However, it is clear the effect of HBD2 is strain dependent, as for instance HBD2 was without effect on the cytotoxic activity of the clinical strain P. fluorescens MFN1032 (Madi et al., 2013). This difference was explained by the fact that clinical strains are adapted to the human host, their virulence being maximal because of their adaptation to a stressing environment. In the present situation, a sub-lethal concentration of HBD2 induced a decrease in MFP05 cytotoxicity. It is important to note that unlike MF37, that was naïve in regard of eukaryotic cells and MFN1032, that was behaving as an opportunistic pathogen, MFP05 was collected on healthy normal skin, and was then adapted to this ecological niche.

In order to be tolerated by the host defense mechanisms, bacteria use to develop escape response by reducing antigens presentation (Van der Aar et al., 2013). In the case of MFP05, this reduction of virulence also appears as an adaptation mechanism aimed at improving skin tolerance and should be considered as the signature of a commensal germ. The absence of effect of LL37 on MFP05 cytotoxicity was confirmed by combination of this peptide with HBD2 since we only observed a decrease of cytotoxicity equivalent to that of HBD2 alone, whereas antimicrobial peptides have generally synergistic activities (Cassone

and Otvos, 2010). However, it is clear that *P.fluorescens* MFP05 was also actually detecting LL37 since this antimicrobial peptide had an important effect, even more pronounced than HDB2, on MFP05 biofilm formation activity. Unlike on P. aeruginosa where it reduced the formation of biofilm (Overhage et al., 2008), on P. fluorescens MFP05 LL37 was stimulating biofilm assembly. Nevertheless, this effect was transient, and not additive with that of HBD2 suggesting that, at least in *P.fluorescens* and at sublethal concentrations as normally present in skin, the two antimicrobial peptides should act by different mechanisms.

The present report is the first one studying the effect of skin peptides on a *P.fluorescens* human skin strain. *P.fluorescens* MFP05 appears to behave as a commensal microorganism, reducing its virulence in response to sublethal concentrations antimicrobial peptides but also capable to increase its invasive or adhesion potential under the effect of a mediator of inflammation such as SP. This strain appears well adapted to the human skin microenvironment suggesting that P. fluorescens should be metabolically active on the skin surface. Interactions of this species with other members of the skin microbiote deserve further investigations.

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