Biofilm 7 (2024) 100206

Contents lists available at ScienceDirect

Biofilm

journal homepage: www.sciencedirect.com/journal/biofilm

Antibiofilm activity of *Prevotella* species from the cystic fibrosis lung microbiota against *Pseudomonas aeruginosa*

Lucia Grassi [a], Kyle L. Asfahl [b], Sara Van den Bossche [a], Ine Maenhout [a], Andrea Sass [a], Yannick Vande Weygaerde [c], Eva Van Braeckel [c,d], Bruno Verhasselt [e,f], Jerina Boelens [e,f], Michael M. Tunney [g], Ajai A. Dandekar [b], Tom Coenye [a], Aurélie Crabbé [a,*]

[a] Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium
[b] Department of Microbiology, University of Washington, Seattle, WA, USA
[c] Cystic Fibrosis Reference Centre, Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium
[d] Department of Internal Medicine and Paediatrics, Ghent University, Ghent, Belgium
[e] Laboratory of Medical Microbiology, Ghent University Hospital, Ghent, Belgium
[f] Department of Diagnostic Sciences, Ghent University, Ghent, Belgium
[g] School of Pharmacy, Queen's University Belfast, Belfast, Northern Ireland, UK

A R T I C L E I N F O

Keywords:
Pseudomonas aeruginosa
Lung microbiota
Cystic fibrosis
Biofilm
Interspecies interactions

A B S T R A C T

It is increasingly recognized that interspecies interactions may modulate the pathogenicity of *Pseudomonas aeruginosa* during chronic lung infections. Nevertheless, while the interaction between *P. aeruginosa* and pathogenic microorganisms co-infecting the lungs has been widely investigated, little is known about the influence of other members of the lung microbiota on the infection process. In this study, we focused on investigating the impact of *Prevotella* species isolated from the sputum of people with cystic fibrosis (pwCF) on biofilm formation and virulence factor production by *P. aeruginosa*. Screening of a representative collection of *Prevotella* species recovered from clinical samples showed that several members of this genus (8 out 10 isolates) were able to significantly reduce biofilm formation of *P. aeruginosa* PAO1, without impact on growth. Among the tested isolates, the strongest biofilm-inhibitory activity was observed for *Prevotella intermedia* and *Prevotella nigrescens*, which caused a reduction of up to 90% in the total biofilm biomass of several *P. aeruginosa* isolates from pwCF. In addition, a strain-specific effect of *P. nigrescens* on the ability of *P. aeruginosa* to produce proteases and pyocyanin was observed, with significant alterations in the levels of these virulence factors detected in LasR mutant strains. Overall, these results suggest that non-pathogenic bacteria from the lung microbiota may regulate pathogenicity traits of *P. aeruginosa*, and possibly affect the outcome of chronic lung infections.

1. Introduction

Despite significant advances in the development of therapies targeting the basic defect in cystic fibrosis (CF), [i.e., cystic fibrosis transmembrane regulator (CFTR) modulators], chronic airway infections remain a leading contributor to lung disease [1]. Currently, more than 40% of adults with CF [2] develop chronic infections with *Pseudomonas aeruginosa* during their lifetime, with detrimental consequences on lung function and life expectancy of these individuals [3]. Although the clinical use of CFTR modulators has led to an overall decrease in *P. aeruginosa* load in the lower respiratory tract [4,5], relapsing and recurring lung infections are commonly seen in people with CF (pwCF) [6–8]. Pre-existing structural airway damage and adaptive behaviour of *P. aeruginosa* are among the main factors predisposing to persistent colonization of the CF lung [9,10]. In this regard, along with inherited mechanisms of antibiotic resistance, the ability of *P. aeruginosa* to form biofilms plays a key role in reducing the effectiveness of current antibiotic therapies and in preventing the eradication of the infection [11–13].

In addition to clinically important pathogens like *P. aeruginosa*, the respiratory tract of pwCF harbours many other microbial species that are mostly regarded as non-pathogenic [14,15]. A growing body of literature has shown that the interaction of *P. aeruginosa* with other microorganisms in the CF airways has the potential to influence the pathogenesis of chronic lung infections [16–18]. Several studies have

* Corresponding author. Ottergemsesteenweg 460, 9000, Ghent, Belgium.
E-mail address: aurelie.crabbe@ugent.be (A. Crabbé).

https://doi.org/10.1016/j.bioflm.2024.100206
Received 24 January 2024; Received in revised form 6 May 2024; Accepted 6 June 2024
Available online 8 June 2024
2590-2075/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

Abbreviations

anBHI	anaerobic Brain Heart Infusion
AUC	Area Under the Curve
CBA	Columbia Blood Agar
CF	Cystic Fibrosis
CFS	Cell-Free Supernatant
CFTR	Cystic Fibrosis Transmembrane Regulator
CFU	Colony-Forming Units
FAA	Fastidious Anaerobe Agar
FEV_1	Forced Expiratory Volume in 1 second
KVLB	Kanamycin-Vancomycin Laked Blood
MALDI-TOF MS	MALDI-TOF Mass Spectrometry
MSP	Main Spectrum Profiles
pwCF	people with Cystic Fibrosis

demonstrated the existence of a wide range of (synergistic and/or antagonistic) interactions between *P. aeruginosa* and other CF pathogens, including *Staphylococcus aureus*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia* and *Aspergillus fumigatus* complex (reviewed by Ref. [19,20]). These interactions have been reported to affect *P. aeruginosa* virulence and/or biofilm formation both *in vitro* and in animal infection models [21–25]. Altered susceptibility of *P. aeruginosa* to different classes of antibiotics (e.g., aminoglycosides and β-lactams) has also been observed in co-culture with *S. aureus*, *S. maltophilia*, *A. fumigatus* and *C. albicans* (reviewed by Ref. [26,27]). While the impact of common respiratory pathogens on the pathogenicity of *P. aeruginosa* has been extensively examined, interspecies interactions involving other members of the CF lung microbiota are still largely unexplored [28].

Anaerobic bacteria have emerged as important members of the CF lung microbiota, with *Prevotella* being the most prevalent and abundant genus detected in respiratory samples [29,30]. Although the prevalence of *Prevotella* species in the CF lower airway is similar to that of *P. aeruginosa* [31,32], their contribution to infection and inflammation remains controversial [33,34]. Several studies have suggested a potential role of these bacterial species in promoting the growth and antibiotic resistance of *P. aeruginosa* through cross-feeding and secretion of antibiotic-degrading enzymes, respectively [35–37]. In contrast, a positive impact of certain *Prevotella* species on lung health has recently been proposed based on their ability to modulate inflammatory responses induced by *P. aeruginosa* or other pro-inflammatory mediators [38,39]. In addition, the abundance of *Prevotella* species and other obligate anaerobes of the CF lung microbiota (e.g., *Veillonella*) has been positively associated with milder lung disease and/or lower levels of pro-inflammatory mediators [15,29,30,40–44].

Considering these contradictory observations, we became interested in understanding if and how *Prevotella* species might influence the progression of lung disease through interactions with key pathogens like *P. aeruginosa*. A better understanding of the impact of these microbiota members on pathogenicity traits of *P. aeruginosa* may help elucidate their role in chronic lung infections. In the present study, we investigated whether various *Prevotella* species commonly detected in the CF lung microbiota exert a direct influence on biofilm formation, growth and production of clinically relevant virulence factors (such as proteases and pyocyanin) by *P. aeruginosa*.

2. Materials and methods

2.1. Bacterial strains and culture conditions

All *P. aeruginosa* and *Prevotella* spp. strains used in the present study are listed in Table 1. The laboratory strain *P. aeruginosa* PAO1 (ATCC 15692) and CF clinical isolates from the international *P. aeruginosa* reference panel (AA2, AMT0023-30, AMT0060-3, IST27, IST27-N, U018a and 2192) were used in this study [45]. Additional CF strains included natural LasR mutants (E113 and E131) and a LasR variant of *P. aeruginosa* AA2, previously obtained through *in vitro* evolution experiments. Sanger sequencing of *lasR* was performed to verify the specific mutation present in the AA2 Δ*lasR* strain, and a single nucleotide deletion was found at position 543 (543delG). For routine cultures, these strains were grown aerobically on Luria-Bertani (LB) agar and in LB broth for 16–18 h at 37°C. All *Prevotella* spp. strains were collected in the present study (as described below) with the exception of *Prevotella oulorum* (Table 1). For the preparation of pure cultures and the enumeration of colony-forming units (CFU), the *Prevotella* spp. strains were cultured on Columbia Blood Agar (CBA; Columbia agar added with 5% sheep blood) under anaerobic conditions (5% H_2, 5% CO_2, 90% N_2; Bactronez-2 Anaerobic Chamber, SHEL-LAB, Cornelius, USA) for 2–3 days at 37°C. Liquid cultures were obtained by growing bacteria in anaerobic Brain Heart Infusion broth (anBHI; BHI containing 50 μg/mL L-cysteine and 1 μg/mL resazurin), until the stationary phase of growth was reached (16–24 h at 37°C, anoxic atmosphere).

Table 1
Overview of strains used in the present study.

Species	Strain	Origin and relevant features
Pseudomonas aeruginosa	PAO1 (ATCC 15692)	Non-CF, wound [46]
	AA2	CF (early isolate) [47]
	AA2 Δ*lasR*	Evolved strain [48]
	AMT0023-30, AMT0060-3	CF (early isolate) [49]
	E113, E131	CF, natural LasR mutants [50]
	IST27	CF, mucoid [51]
	IST27-N	Spontaneous non-mucoid variant of IST27 [51]
	U018a	CF [52]
	2192	CF (late isolate) [53]
Prevotella denticola	P10_ANAE4	CF sputum
Prevotella histicola	P5_ANAE4	CF sputum
Prevotella intermedia	CFI0117	CF sputum
Prevotella jejuni	P8_ANAE11	CF sputum
Prevotella melaninogenica	P4_ANAE10	CF sputum
Prevotella nigrescens	P4_ANAE1	CF sputum
Prevotella oris	P3_ANAE1	CF sputum
Prevotella oulorum	B028_V3_SPU_E1K	CF sputum
Prevotella pallens	P15_ANAE23	CF sputum
Prevotella salivae	P1_ANAE3	CF sputum

Abbreviation – CF: cystic fibrosis.

2.2. Clinical data and sputum sample collection

Sputum samples were obtained from 15 pwCF attending the outpatient clinic of Ghent University Hospital, after having received approval of the local ethics committee (registration number: B670201836204). Inclusion criteria for participants were ability to produce sputum, chronic colonization with *P. aeruginosa* (based on *P. aeruginosa*-positive culture in >50% of samples taken in the previous 12 months) and being older than 12 years. The main clinical features of the study cohort are summarized in Table S1. These include: age, gender, *cftr* gene mutation, lung function (measured as forced expiratory volume in 1 second, FEV_1%), presence of pancreatic insufficiency, duration of *P. aeruginosa* colonization, and type of treatment at the time of sampling (i.e., CFTR modulator and antibiotic therapies). Sputum samples were obtained through spontaneous expectoration and processed within 2 h of collection, as previously described [39].

2.3. Isolation and identification of Prevotella species from CF sputum samples

Isolation of *Prevotella* species was performed by culturing serial dilutions of the sputum on different media under anaerobic conditions for 5 days at 37°C. The following culture media were selected based on previous successful isolation of *Prevotella* species [54,55]: CBA, Fastidious Anaerobe Agar (FAA) and Kanamycin-Vancomycin Laked Blood agar (KVLB; tryptic soy agar added with 0.1 μg/mL kanamycin, 7.5 μg/mL vancomycin, 10 μg/mL vitamin K_1, 0.05 ng/mL hemin and 5% laked horse blood). For each sample, 15 to 30 morphologically distinct colonies were purified through serial passages on the same medium used for their initial isolation. Identification of these isolates was performed by MALDI-TOF mass spectrometry (MALDI-TOF MS; Bruker Microflex, Bruker Daltonics, Bremen, Germany) due to the ability of this technology to provide a rapid and accurate species-level identification of anaerobic bacteria (including members of the *Prevotella* genus) [56,57]. The MALDI Biotyper software (version 4.1) (Bruker Daltonics) was used for analysing and comparing the mass spectra derived from each unknown isolate with the reference spectra in the database (MALDI Biotyper IVD Library Revision J) (updated with 11758 MSP). Results of the MALDI-TOF MS analysis were expressed as score with values ranging from 0 to 3, where scores $\geq$ 2.0 and $\geq$ 1.7 were set as a threshold for the identification of isolates at the species and genus level, respectively. The identification was considered not reliable at score values below 1.7 [30,58].

2.4. Preparation of cell-free supernatants (CFS) of Prevotella species

One representative isolate for each *Prevotella* species identified (referred to as *Prevotella* spp. strain) was selected for the study. Stationary-phase cultures of the tested *Prevotella* spp. strains were centrifuged at 4500×*g* for 10 min, and supernatants were sterilized by filtration using 0.22 μm hydrophilic polyethersulfone filters (Millex-GP, Merck Millipore, Burlington, Massachusetts, USA). The CFU/mL of bacterial cultures was determined prior to the collection of the supernatants by plating. Depending on the tested strain, average CFU counts ranging from 1.5×10^8 to 2.9×10^9 CFU/mL were reached in stationary-phase cultures (Fig. S1). The pH of the CFS was measured and adjusted to the value obtained for anBHI alone (6.95 ± 0.10).

2.5. Assessment of P. aeruginosa growth

The antibacterial activity of *Prevotella* spp. strains was assessed by monitoring the growth of *P. aeruginosa* PAO1 in the presence and absence of the CFS. To this end, PAO1 overnight cultures were centrifuged and bacterial cells were resuspended in 4-fold concentrated BHI (BHI 4X) at a final density of 5×10^5 CFU/mL. Concentrated BHI was used for the preparation of the *P. aeruginosa* PAO1 suspension to compensate for a possible reduction in nutrient availability in the CFS due to the growth of *Prevotella* species [59]. A volume of 40 μL of PAO1 suspension in BHI 4X was added to 160 μL of the CFS obtained from individual *Prevotella* spp. strains into the wells of a U-bottom 96-well plate. Hence, the CFS was tested at the final concentration of 80% (v/v). For the growth control, bacteria were added to fresh anBHI. Growth of *P. aeruginosa* PAO1 was determined by measuring the optical density at 590 nm (OD_{590}) every 30 min for 24 h using a microplate reader (VICTOR Nivo; PerkinElmer, Waltham, Massachusetts, USA). Area Under the Curve (AUC) was calculated for each growth dataset using GraphPad Prism 9 (GraphPad Software, San Diego, California, USA). To estimate kinetic growth parameters (lag phase duration, maximum growth rate during the logarithmic phase and maximum culture density), a three-parameter Gompertz equation was fitted to the growth data using SigmaPlot 15 (SYSTAT Software, Chicago, Illinois, USA) [60].

2.6. Assessment of P. aeruginosa biofilm formation

The antibiofilm potential of *Prevotella* spp. strains was evaluated based on their ability to prevent biofilm formation of *P. aeruginosa* PAO1 and clinical CF strains. As described above, a volume of 40 μL of bacterial suspension (5×10^5 CFU/mL in BHI 4X) was added to 160 μL of CFS or anBHI (untreated control for biofilm formation) into U-bottom 96-well plates. For selected *Prevotella* spp. strains, serial dilutions of the CFS were performed in anBHI [i.e., final concentration of the CFS ranging between 0.02 and 20% (v/v)] and tested for their biofilm-inhibitory activity. After 24 h of incubation (37°C, 100 rpm), biofilm biomass was assessed through a standard crystal violet staining [61]. In parallel, the number of culturable biofilm-forming bacteria was determined by plating [62]. To this end, biofilms were washed twice with physiological saline (0.9% NaCl) to remove non-adherent cells and disrupted through two consecutive cycles of vortexing (900 rpm, 5 min) and sonication (4 kHz, 5 min; Branson Ultrasonic Bath 3510, Branson Ultrasonics, Danbury, Connecticut, USA). Biofilm-associated bacteria were serially diluted and plated on LB agar (detection limit: 10^2 CFU/mL). For selected conditions, the number of non-adherent (planktonic) cells was also assessed. The number of CFU was quantified after aerobic incubation of the plates for 16–18 h at 37°C.

2.7. Quantification of P. aeruginosa virulence factors

The effect of selected *Prevotella* spp. strains on the production of virulence factors by *P. aeruginosa* (PAO1 and CF strains) was assessed based on the levels of total proteases and pyocyanin. To this end, *P. aeruginosa* suspensions (400 μL, 5×10^5 CFU/mL in BHI 4X) were inoculated into 1.6 mL of CFS of the *Prevotella* spp. strains or anBHI (untreated control) in 24-well plates. Following incubation (24 h at 37°C, 100 rpm), bacterial cultures were centrifuged and the supernatant filter-sterilized. An azocasein assay was performed to evaluate the proteolytic activity of *P. aeruginosa*, while pyocyanin levels in the filtered supernatant were quantified by spectrophotometry following chloroform-HCl extraction [63]. The proteolytic activity of the CFS alone was also measured under the same experimental conditions. To account for subtle differences in growth between samples, absorbance values obtained for the quantification of proteases (ABS_{420}) and pyocyanin (ABS_{530}) were normalized to the number of viable bacteria (Log CFU mL^{-1}) at the end of the incubation period.

2.8. Statistical analysis

All experiments were performed at least in biological triplicate. Statistical analysis was carried out using SPSS statistics software, version 28 (SPSS, Chicago, Illinois, USA). Normal distribution of the data was verified using the Shapiro-Wilk test. For normally distributed data, differences between mean values were evaluated with a *t*-test for independent samples in case of two-sample comparison and a one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test in case of multiple comparison. Non-normally distributed data were analysed using the Mann-Whitney test or the Kruskal-Wallis test followed by Dunn post hoc test. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Representative Prevotella species of the lung microbiota were successfully isolated from CF sputum

Sputum samples for bacterial isolation were collected from 15 pwCF chronically colonized with *P. aeruginosa*. Most of pwCF (13 out of 15) were treated with antibiotics at the time of sampling, while only a few (3 out of 15) received CFTR modulator therapy (Table S1). Regardless of ongoing treatments, we were able to isolate a total of 9 different species

of the *Prevotella* genus from these clinical samples (Fig. 1A, Table S2). A large proportion of the samples were positive for the presence of *Prevotella* species, with up to 3 different species detected from each patient (Fig. 1B). In addition to *Prevotella*, anaerobic culture of sputum resulted in the isolation of 11 other genera (26 bacterial species in total), which included both predominant and low-abundance anaerobes that are often detected in pwCF [30] (Fig. 1A, Table S2). While *Streptococcus* and *Veillonella* species were often isolated from sputum samples, the isolate collection was dominated by members of the *Prevotella* genus, which counted the highest number of distinct species identified across sputum samples (Fig. 1A, Table S2). With the exception of *P. jejuni*, all *Prevotella* species isolated in this study represent a significant part of those more commonly found in the CF lung microbiota [54,64]. Growth of these species on blood-containing medium also revealed the presence of recurrent phenotypic variants, such as dark- or black-pigmented strains of *P. histicola*, *P. intermedia* and *P. nigrescens* (Fig. S2).

3.2. Members of the Prevotella genus inhibit P. aeruginosa biofilm formation in vitro

We evaluated whether strains belonging to ten common *Prevotella* species from the CF lung microbiota could influence the *in vitro* biofilm formation of the reference strain *P. aeruginosa* PAO1. Given the complexity of establishing co-cultures of *P. aeruginosa* and anaerobic bacteria due to their different growth requirements, we exposed *P. aeruginosa* PAO1 to the CFS obtained from stationary-phase cultures of individual *Prevotella* spp. strains. The use of spent media is relevant to the type of pathogen-microbiota interactions that occur in the CF lung, where *P. aeruginosa* has been shown to mainly form monospecies biofilms [65,66]. Hence, secreted effector molecules from *Prevotella* species and other members of the lung microbiota are likely to mediate interspecies interactions in the CF lung environment. Growth of *P. aeruginosa* PAO1 in the presence and absence of the CFS was assessed in parallel to determine if any effect on biofilm formation was related to a change in bacterial density.

Among the tested *Prevotella* spp. strains, we observed a variable effect on *P. aeruginosa* biofilm formation (Fig. 2). In particular, the CFS of 8 out of 10 tested strains caused a statistically significant reduction (45–80%) in the total biofilm biomass as compared to the untreated control. Only *P. oulorum* and *P. salivae* had no significant effect on biofilm formation. The strongest biofilm-inhibitory activity was exerted by the CFS of the *P. intermedia*, *P. jejuni* and *P. nigrescens* strains tested, with all three causing a decrease of around 80% in biofilm formation by *P. aeruginosa* PAO1 (Fig. 2). Importantly, none of these three strains exhibited antibacterial activity under the tested conditions, as no major changes in growth were detected when *P. aeruginosa* PAO1 was cultured in the presence of their CFS (Table 2). Nevertheless, some of the tested *Prevotella* spp. strains (4 out of 10 tested strains) significantly affected at least one of the growth parameters of *P. aeruginosa* PAO1 (Table 2). Notably, all *Prevotella* spp. strains caused a statistically significant increase in growth rate compared to the untreated control. However, such an increase in growth rate did not correspond to major changes in bacterial density, thus suggesting minimal impact of this effect on overall growth (Table 2).

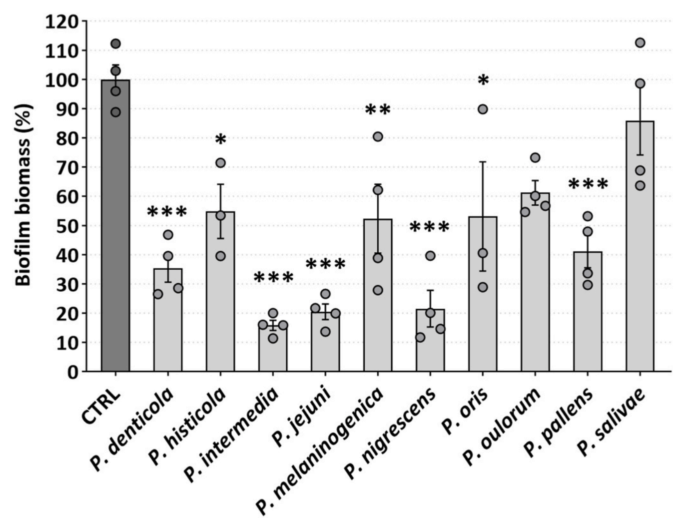

Fig. 2. Effect of different *Prevotella* spp. strains on biofilm formation by *P. aeruginosa* PAO1. Total biofilm biomass of *P. aeruginosa* was assessed by crystal violet staining after 24 h of incubation in the presence and absence of the CFS [80% (v/v)] obtained from *Prevotella* species. Data are reported as percentage of biofilm biomass relative to the untreated control ± standard error ($n \geq 3$). Circles represent individual data points. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to CTRL (one-way ANOVA followed by Bonferroni post hoc test). CTRL: untreated *P. aeruginosa* PAO1 biofilm.

For selected *Prevotella* spp. strains, we also tested increasing concentrations of the CFS for their ability to effectively reduce biofilm formation of *P. aeruginosa* PAO1. To this end, *P. intermedia* and *P. nigrescens* were chosen given their potent antibiofilm activity against *P. aeruginosa* PAO1 (Fig. 2) and their high prevalence in the CF lung amongst *Prevotella* species [54,64]. Interestingly, a statistically significant reduction in the biofilm biomass was observed at concentrations up to 0.02% (v/v) and 5% (v/v) of the CFS obtained from *P. intermedia* and *P. nigrescens*, respectively (Fig. 3). Biofilm formation of *P. aeruginosa*

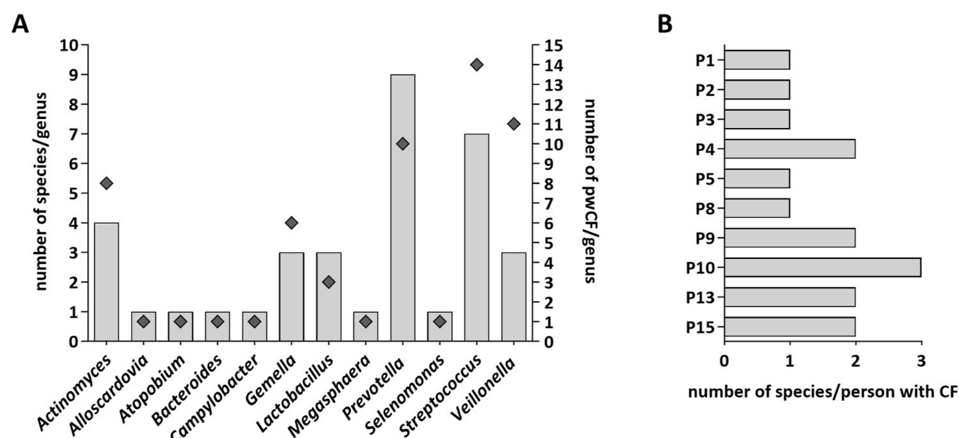

Fig. 1. Isolation of bacteria from the CF lung microbiota under anaerobic conditions. A) Overview of bacterial genera isolated from CF sputum under anaerobic growth conditions. Bars: number of bacterial species identified for each genus (left y axis). Diamonds: number of pwCF harboring microbiome members belonging to the identified genera (right y axis). **B)** Number of distinct *Prevotella* species isolated from each person living with CF. P: person living with CF (e.g., P1: person 1).

Table 2
Effect of different *Prevotella* spp. strains on the growth of *P. aeruginosa* PAO1. Growth of PAO1 in the presence and absence of the CFS [80% (v/v)] obtained from *Prevotella* spp. strains was evaluated by determining the AUC and growth parameters (lag phase duration, growth rate and maximum culture density) from OD_{590} measurements. Data are reported as average ± standard error of at least three biological replicates. *p < 0.05, **p < 0.01, ***p < 0.001 compared to the untreated control (independent samples *t*-test or Mann-Whitney test). CTRL: untreated PAO1.

	AUC	Lag phase (h)[a]	Growth rate (h^{-1})[a]	OD_{max}[a]
CTRL	**24.3 ± 0.4**	**7.83 ± 0.06**	**0.47 ± 0.03**	**1.75 ± 0.02**
P. denticola	23.2 ± 0.5	8.00 ± 0.06	0.59 ± 0.01 (***)	1.71 ± 0.02
P. histicola	22.9 ± 0.3 (*)	8.59 ± 0.12 (***)	0.62 ± 0.01 (***)	1.70 ± 0.01 (*)
P. intermedia	24.7 ± 0.2	8.01 ± 0.20	0.58 ± 0.02 (*)	1.77 ± 0.01
P. jejuni	24.2 ± 0.1	8.14 ± 0.02	0.61 ± 0.01 (***)	1.74 ± 0.01
P. melaninogenica	24.3 ± 0.2	8.11 ± 0.04	0.62 ± 0.01 (***)	1.72 ± 0.01
P. nigrescens	24.1 ± 0.1	7.99 ± 0.08	0.59 ± 0.01 (**)	1.75 ± 0.01
P. oris	22.6 ± 0.1 (**)	8.61 ± 0.08 (***)	0.59 ± 0.01 (**)	1.68 ± 0.01 (**)
P. oulorum	24.0 ± 0.2	8.17 ± 0.07	0.59 ± 0.01 (***)	1.73 ± 0.01
P. pallens	23.1 ± 0.3 (*)	8.24 ± 0.06 (*)	0.59 ± 0.01 (***)	1.69 ± 0.01 (**)
P. salivae	22.3 ± 0.2 (**)	8.51 ± 0.05 (***)	0.63 ± 0.02 (**)	1.66 ± 0.01 (***)

Abbreviations – AUC: area under the curve; OD_{max}: maximum optical density.
[a] Growth parameter at 16 h of growth (i.e., early stationary phase).

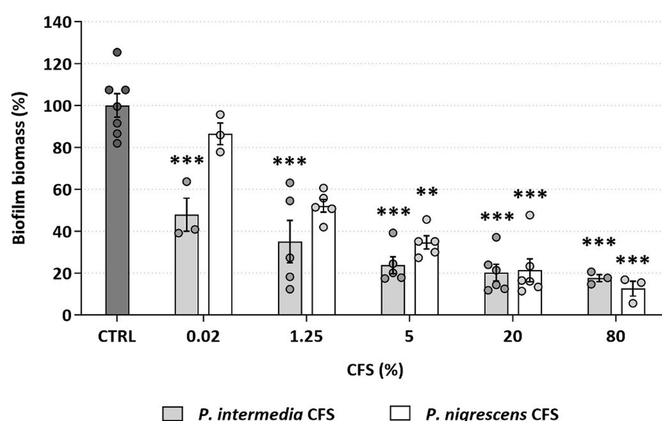

Fig. 3. Effect of different concentrations of *P. intermedia* and *P. nigrescens* CFS on biofilm formation by *P. aeruginosa* PAO1. Total biofilm biomass of *P. aeruginosa* was assessed by crystal violet staining after 24 h of incubation in the presence and absence of different concentrations of the CFS [0.02–80% (v/v)] obtained from *P. intermedia* and *P. nigrescens*. Data are reported as percentage of biofilm biomass relative to the untreated control ± standard error (n ≥ 3). Circles represent individual data points. **p < 0.01, ***p < 0.001 compared to CTRL (one-way ANOVA followed by Bonferroni post hoc test or Kruskal-Wallis test followed by Dunn post hoc test). CTRL: untreated *P. aeruginosa* PAO1 biofilm.

PAO1 was decreased by more than 50% in the presence of these concentrations of the CFS. While the CFS of both strains exerted a comparable effect at the highest concentrations tested [i.e., up 1.25% (v/v)], a stronger biofilm-inhibitory activity of *P. intermedia* compared to *P. nigrescens* was evident at lower concentrations of the CFS [i.e., 0.02% (v/v), $p = 0.015$] (Fig. 3).

3.3. *P. intermedia* and *P. nigrescens* reduce biofilm formation of a broad panel of *P. aeruginosa* CF strains

Next, we evaluated whether the tested strains of *P. intermedia* and *P. nigrescens* could also inhibit biofilm formation of a panel of *P. aeruginosa* isolates from pwCF. In these experiments, *P. aeruginosa* biofilm formation in the presence and absence of the CFS [at the highest concentration of 80% (v/v)] was evaluated in terms of both total biofilm biomass and number of biofilm-forming bacteria (based on CFU counts).

The CFS of *P. intermedia* and *P. nigrescens* significantly reduced biofilm formation of all *P. aeruginosa* CF strains, except AMT0060-03 and IST27 (Fig. 4A). Depending on the tested *P. aeruginosa* strain, the percentage reduction in biofilm biomass ranged from 40 to 90% compared to the untreated control. No significant differences were found between the CFS of *P. intermedia* and *P. nigrescens* in inhibiting biofilm formation (Fig. 4A). Interestingly, the biofilm-inhibitory effect of the CFS was observed against most intermediate (i.e., A_{580} 0.35 to 0.95; [67]) and all strong (i.e., $A_{580} > 0.95$) biofilm producers. Thus, one explanation for the lack of activity of the CFS against *P. aeruginosa* AMT0060-03 and IST27 might be the overall poor biofilm formation capacity of these strains (i.e., $A_{580} < 0.35$ and $A_{580} < 0.65$, respectively). For the majority of *P. aeruginosa* CF strains, the decrease observed in total biofilm biomass did not correspond to a reduction in the number of biofilm-forming bacteria (Fig. 4B). Indeed, the number of biofilm-forming bacteria was significantly decreased in only 2 out of 11 *P. aeruginosa* strains (i.e., PAO1 and AMT0023-30). Exposure to the CFS of *P. intermedia* and *P. nigrescens* resulted in a reduction of 0.3–0.6 Log and 1 Log in CFU counts of *P. aeruginosa* AMT0023-30 and PAO1, respectively (Fig. 4B). For these strains, the number of viable bacteria in the planktonic fraction was also assessed in order to verify whether the observed decrease in CFU counts was due to actual inhibition of biofilm formation rather than a growth-inhibitory effect of the CFS. A statistically significant reduction in the number of non-adherent (planktonic) bacteria in the presence of the CFS was noticed for both *P. aeruginosa* PAO1 (i.e., 0.2 to 0.3 Log) and AMT0023-30 (i.e., 0.3 Log) (Fig. S3). The observed minor effect of the CFS on planktonic cells could be reflected in the equally small reduction of biofilm-forming bacteria for *P. aeruginosa* AMT0023-30 (Fig. 4B). However, the considerable decrease in biofilm formation observed for *P. aeruginosa* PAO1 cannot be fully explained by the minor growth-inhibitory effect of the CFS on the planktonic fraction (Fig. 4B), being in accordance with the growth data where no effect was observed (Table 2).

3.4. Biofilm inhibition correlates with a visible decrease in *P. aeruginosa* aggregation

A qualitative evaluation of the antibiofilm activity of *P. intermedia* and *P. nigrescens* was performed by monitoring the formation of pellicle and macroscopic aggregates of *P. aeruginosa* in the cultures. When cultured in the presence of the CFS of *P. intermedia* and *P. nigrescens*, the reference strain PAO1 did not form visible clusters at the air-liquid interface (Fig. 5A). As a control, we tested *Prevotella* spp. strains that did not exert biofilm-inhibitory activity based on the above-described quantitative assay (i.e., *P. oulorum* and *P. salivae*) (Fig. 2). Macroscopic bacterial aggregates were formed following the treatment with the CFS obtained from these two strains (Fig. 5A). The antibiofilm activity of the CFS of *P. intermedia* and *P. nigrescens* at the macroscopic level was also confirmed for several *P. aeruginosa* CF strains, including AA2, AA2

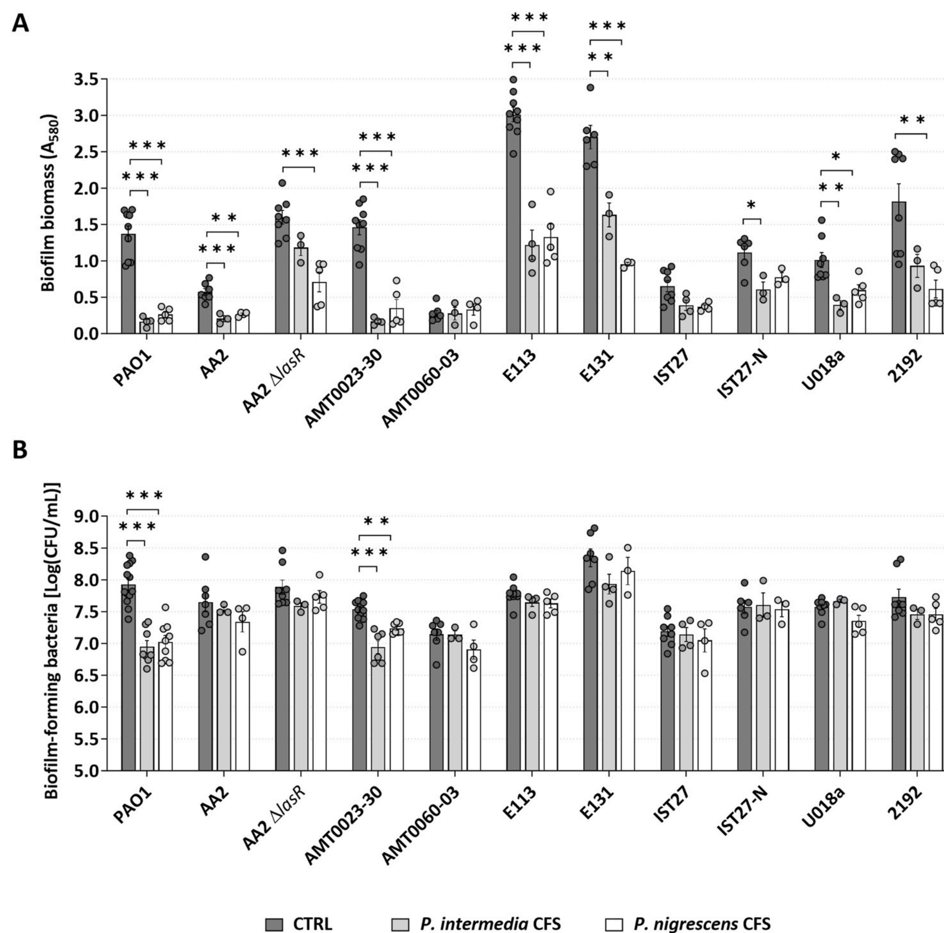

Fig. 4. Effect of *P. intermedia* and *P. nigrescens* on biofilm formation by *P. aeruginosa* CF strains. A) Total biofilm biomass as assessed by crystal violet staining. **B)** Number of biofilm-forming bacteria as assessed by plating and CFU counting. Both biofilm biomass and number of biofilm-forming bacteria were determined after a 24-h exposure of *P. aeruginosa* to 80% (v/v) CFS of *P. intermedia* and *P. nigrescens*. Data are average ± standard error of at least three biological replicates. Circles represent individual data points. *p < 0.05, **p < 0.01, ***p < 0.001 (one-way ANOVA followed by Bonferroni post hoc test). CTRL: untreated *P. aeruginosa*.

Δ*lasR*, AMT0023-30, E113 and E131. Indeed, a considerable decrease in pellicle formation and/or aggregation of all these strains was observed in the presence of the CFS compared to the untreated control (Fig. 5B). Although less evident compared to the above-mentioned strains, some differences in aggregation were noticed between untreated and CFS-treated samples of *P. aeruginosa* U018a and 2192 (Fig. 5B).

3.5. P. intermedia and P. nigrescens do not have a major impact on P. aeruginosa virulence factor production

Finally, we explored whether *P. intermedia* and *P. nigrescens* could also influence other phenotypic traits that are associated with the pathogenicity of *P. aeruginosa* during chronic lung infections. We focused on the production of clinically relevant virulence factors, such as proteases and pyocyanin [68,69]. In addition, since these *Prevotella* species have previously been reported to produce proteases [70–72], we also assessed the proteolytic activity of the CFS alone under the same experimental conditions.

The production of proteases was significantly decreased by the CFS of *P. intermedia* and/or *P. nigrescens* for only 2 out of 11 *P. aeruginosa* strains (i.e., AA2 Δ*lasR* and E113) (Fig. 6A). In particular, we observed a 2 to 5-fold reduction in proteolytic activity of *P. aeruginosa* AA2 Δ*lasR* in the presence of the CFS of *P. intermedia* and *P. nigrescens*, respectively. In the case of *P. aeruginosa* E113, the CFS of *P. nigrescens* caused a decrease in protease activity of approximately 3.5 times compared to the control (Fig. 6A). As for the CFS alone, background levels of proteolytic activity were detected for *P. nigrescens*, while only a minimal production of proteases was found for *P. intermedia* (Fig. S4). Regarding the levels of pyocyanin, exposure to *P. nigrescens* CFS led to a small but significant induction of pyocyanin production in two of the tested *P. aeruginosa* strains (i.e., AA2 Δ*lasR* and E131) (Fig. 6B). Specifically, a 1.7–2.5-fold increase in pyocyanin production was detected for *P. aeruginosa* E131 and AA2 Δ*lasR*, respectively (Fig. 6B).

4. Discussion

Although numerous studies have demonstrated the ability of anaerobic bacteria to colonize the respiratory tract of pwCF, their potential role (either beneficial, detrimental, or neutral) in disease progression remains unclear [33,34]. The load of certain anaerobic species has been shown to be comparable to that of conventional respiratory pathogens [30,31,54], thus supporting the likelihood that interspecies interactions occur. Such interactions have been proposed to impact the course of lung infections, possibly influencing the severity and outcome of CF lung disease. Nevertheless, there is limited knowledge on how anaerobic bacteria of the lung microbiota may influence the behaviour of CF pathogens [27,73].

Considering their high abundance in CF airway secretions, we focused on investigating the influence of *Prevotella* species on pathogenicity traits of *P. aeruginosa* that are relevant to chronic lung infections. We found that different *Prevotella* spp. strains were able to significantly decrease *in vitro* biofilm formation of *P. aeruginosa*, without a major impact on growth. The strongest biofilm-inhibitory activity against *P. aeruginosa* PAO1 was observed for the tested strains of *P. intermedia*,

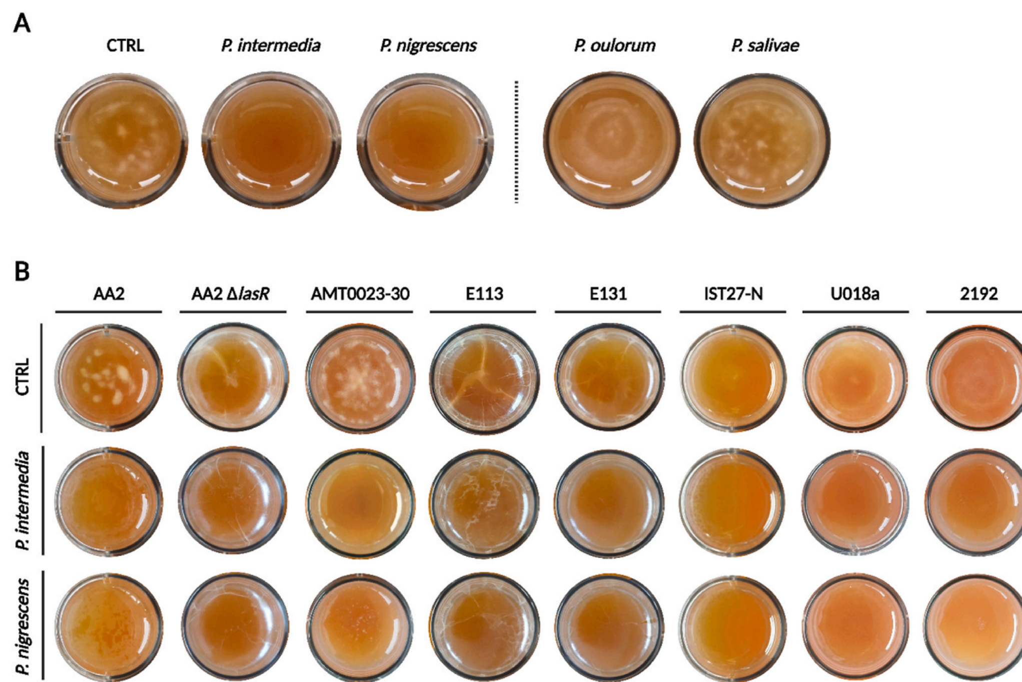

Fig. 5. Macroscopic images of a multiwell plate with *P. aeruginosa* exposed to the CFS of *Prevotella* spp. strains. A) Representative images of macroscopic aggregates formed by the reference strain PAO1 in the presence and absence of the CFS [80% (v/v)] of different *Prevotella* spp. strains after 24 h of incubation. **B)** Representative images of *P. aeruginosa* CF strains cultured in the presence of the CFS [80% (v/v)] of *P. intermedia* and *P. nigrescens* for 24 h. CTRL: untreated *P. aeruginosa*. Diameter of the well: 14 mm.

P. nigrescens and *P. jejuni*, with a reduction of at least 80% in total biofilm biomass. A similar effect of *P. intermedia* and *P. nigrescens* was confirmed against a diverse panel of *P. aeruginosa* CF strains, thus indicating a broad-spectrum antibiofilm activity of these *Prevotella* spp. strains. Interestingly, the CFS obtained from both *P. intermedia* and *P. nigrescens* was effective in reducing biofilm formation at very low concentrations [up to 0.02% (v/v)]. While the antibiofilm activity of *Prevotella* species has not been previously reported, a few studies are available on the influence of other anaerobic species of the CF lung microbiota on *P. aeruginosa* biofilm formation. Pustelny et al. [74] demonstrated the ability of *Veillonella parvula* to promote *P. aeruginosa* aggregation *in vitro* and enhance colonization in a murine tumor model. Also, fermentation products generated by facultative anaerobes (e.g., *Streptococcus* and *Rothia* species) have been shown to increase *in vitro* biofilm formation and reduce the clearance of *P. aeruginosa* from the respiratory tract of a murine infection model [75–77]. On the other hand, recent research has revealed a potential protective role of some microbiome members against *P. aeruginosa* infection. For instance, although unable to inhibit adhesion to airway epithelial cells, *Streptococcus salivarius* reduced *P. aeruginosa* burden in the lungs of a rat co-infection model [78]. Together with our observations, these studies underline a different (potentially opposite) role of non-pathogenic members of the CF lung microbiota in the infection process of *P. aeruginosa*.

Interestingly, we observed that the exposure of most *P. aeruginosa* CF strains to the CFS of *P. intermedia* and *P. nigrescens* caused a significant reduction in the total biofilm biomass and overall degree of aggregation, but not in the number of biofilm-forming bacteria. A considerable decrease in the number of adherent bacteria was detected only for *P. aeruginosa* PAO1, while it was minimal in the case of *P. aeruginosa* AMT0023-30. Although more in-depth studies are needed to uncover the mechanism(s) of *P. aeruginosa* biofilm inhibition and identify antibiofilm effector molecule(s), it is possible that the tested *Prevotella* spp. strains could influence the production and/or stability of the biofilm extracellular matrix. Considering the crucial role of the extracellular matrix in the tolerance of biofilms to antimicrobial treatment [12,79], a potential matrix-inhibiting or degrading effect of *Prevotella* species might be of clinical relevance. In this regard, recent studies have reported the ability of different bacteria to reduce the biofilm matrix of *P. aeruginosa*, resulting in improved efficacy of currently available antibiotics. For example, some lactobacilli produce matrix-degrading enzymes [80], which could favour the diffusion of antibiotics through the biofilm layers and their interaction with biofilm-embedded cells [81]. Similarly, enzymatic disruption of the extracellular matrix of *P. aeruginosa* biofilms by the extracellular levanase of *Bacillus subtilis* and the alginate lyase of marine bacteria has been shown to boost the antibiofilm activity of several antibiotics against *P. aeruginosa* [82,83].

In addition to biofilm formation, the production of secreted virulence factors also plays an important role during chronic lung infections by *P. aeruginosa* [68,69]. Here, we found that *P. nigrescens* exerts a strain-dependent effect on the production of proteases and pyocyanin by *P. aeruginosa*. Interestingly, all three *P. aeruginosa* strains that showed altered levels of virulence factor production in response to *Prevotella* spp. strains were LasR mutants (i.e., AA2 Δ*lasR*, E113 and E131). LasR-defective variants are common among *P. aeruginosa* CF isolates and are known to play important roles in the pathogenesis and progression of chronic lung disease [50,84,85]. More research is needed to elucidate the mechanistic basis for this observation. Nevertheless, increased production of pyocyanin by *P. aeruginosa* LasR mutants has already been observed in mixed communities containing *P. melaninogenica,* where it has been shown to drive tolerance to tobramycin [86]. While no prior studies have investigated their direct impact on *P. aeruginosa* virulence, *Prevotella* species have been reported to produce the signalling metabolite autoinducer-2 (AI-2) [54]. Interspecies communication mediated by this molecule has been demonstrated to modulate the expression of different *P. aeruginosa* virulence-associated genes, including *lasB* and phenazine biosynthesis genes [87]. Other non-pathogenic members of the lung microbiota have been shown to influence *P. aeruginosa* virulence both *in vitro* and *in vivo.* For instance, oral streptococci (belonging to the *Streptococcus anginosus* group) were able to stimulate pyocyanin and/or elastase production in co-culture with *P. aeruginosa* CF isolates, including LasR mutants and mucoid strains [88,89]. Increased expression of *P. aeruginosa* virulence factor genes upon exposure to oral

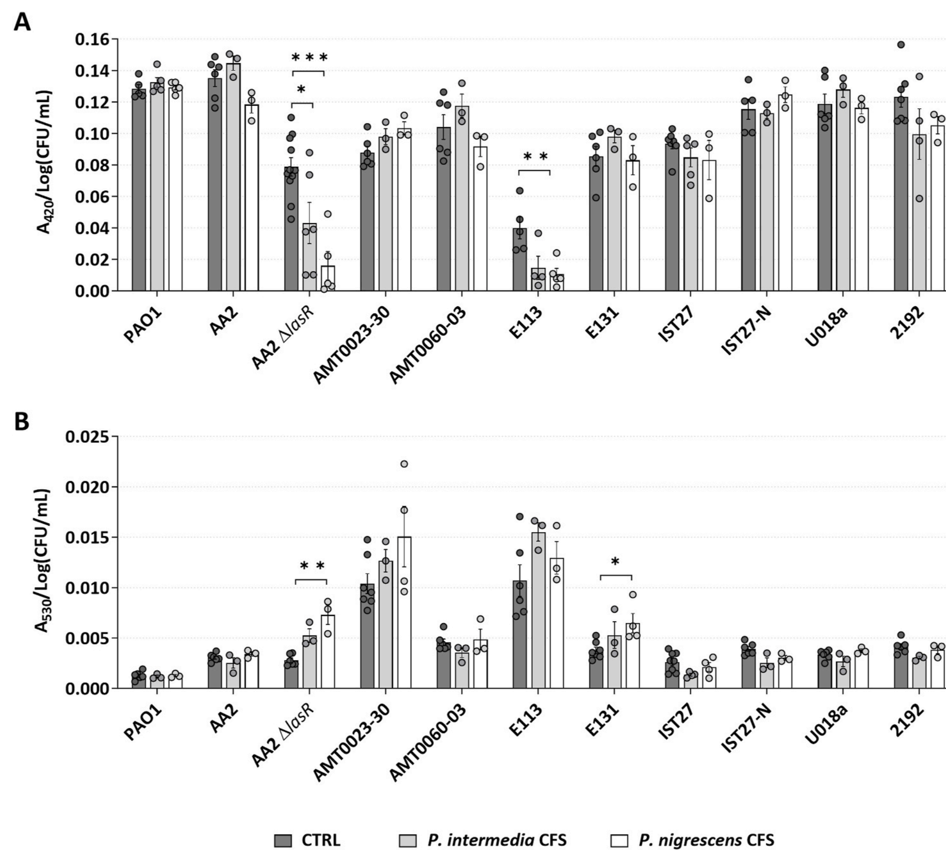

Fig. 6. Effect of *P. intermedia* and *P. nigrescens* on virulence factor production by *P. aeruginosa* PAO1 and CF strains. A) Proteolytic activity as assessed through an azocasein assay. **B)** Pyocyanin production as assessed following chloroform-HCl extraction. Protease activity and pyocyanin production were quantified after a 24-h exposure of *P. aeruginosa* to 80% (v/v) CFS of *P. intermedia* and *P. nigrescens*. Data are average ± standard error of at least three biological replicates. Circles represent individual data points. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (one-way ANOVA followed by Bonferroni post hoc test or Kruskal-Wallis test followed by Dunn post hoc test). CTRL: untreated *P. aeruginosa*.

streptococci was also observed *in vivo* using a *Drosophila melanogaster* infection model [90]. Analogously, co-infection of a rat model with *P. aeruginosa* and a *Streptococcus* strain isolated from CF sputum resulted in enhanced lung damage compared to the mono-infection [87]. In addition, the above-mentioned fermentation products of facultative anaerobes (e.g., 2,3-butanedione) have been reported to induce *in vitro* production of pyocyanin and exotoxin by *P. aeruginosa* [75,77].

Although this study has allowed to explore the influence of *Prevotella* species on different factors contributing to the infection process of *P. aeruginosa* (i.e., biofilm formation and virulence factors), it is also worth mentioning some limitations of the experimental design and possible directions for future research. First, the evaluation of *Prevotella* species for their antibiofilm activity against *P. aeruginosa* was limited to a single strain for each tested species. It is therefore not possible to establish whether the observed biofilm-inhibitory effect is specific to the species or to the particular strain tested, especially considering the strain-level diversity detected within *Prevotella* species [91]. To this purpose, further experiments should be conducted to test multiple strains of the different *Prevotella* species examined in the present study. Furthermore, while the focus of this study was on biofilm formation, it would also be important to investigate the effect of *Prevotella* spp. strains on preformed biofilms of *P. aeruginosa* to obtain a more complete picture of their impact on the persistence of lung infections. More insights on the (lack of) activity of *Prevotella* spp. strains against preformed biofilms could also help to better understand the mechanism(s) underlying their biofilm-inhibitory effect and/or identify their actual target(s). Finally, it would be worth confirming the findings obtained in this study under more physiologically relevant conditions. In this regard, several *in vivo*-like models are currently available that accurately replicate key aspects of *P. aeruginosa* chronic lung infections, including artificial sputum media, three-dimensional cell cultures and *ex vivo* lung tissue explants. Although still representing a simplified version of the *in vivo* microenvironment of the (infected) airways, these model systems could be a useful platform to further investigate the contribution of *Prevotella* species to infection.

In conclusion, our findings demonstrate that members of the *Prevotella* genus affect *P. aeruginosa* biofilm formation, most likely by interfering with cell aggregation and/or extracellular matrix production. Although further research is required to gain a better understanding of the mechanisms underlying the observed biofilm-inhibitory activity, these findings indicate a possible role of *Prevotella* species in the pathophysiology related to *P. aeruginosa* in the CF setting.

CRediT authorship contribution statement

Lucia Grassi: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Kyle L. Asfahl:** Writing – review & editing, Resources, Investigation. **Sara Van den Bossche:** Investigation. **Ine Maenhout:** Investigation. **Andrea Sass:** Writing – review & editing. **Yannick Vande Weygaerde:** Resources. **Eva Van Braeckel:** Writing – review & editing, Resources. **Bruno Verhasselt:** Writing – review & editing, Resources. **Jerina Boelens:** Writing – review & editing, Resources. **Michael M. Tunney:** Writing – review & editing, Resources. **Ajai A. Dandekar:** Writing – review & editing, Resources, Conceptualization. **Tom Coenye:** Writing – review & editing, Conceptualization. **Aurélie Crabbé:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Aurelie Crabbe reports financial support was provided by Ghent University Special Research Fund (BOF/STA/201909/028) and by the Fund Alphonse and Jean Forton of the Kind Baudouin Foundation and the Belgian Cystic Fibrosis Association (2023-J1810150-232649). Lucia Grassi reports financial support was provided by Ghent University Special Research Fund (BOF.DPO.2019.023.01) and Research Foundation Flanders (FWO) (12X6322N). Tom Coenye is senior editor of the journal 'Biofilm'. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank the technical staff at the Laboratory of Medical Microbiology of Ghent University Hospital (Jen Vandevijver, Joris Van Caenegem and Nathaniel Deloof) for providing assistance with MALDI-TOF MS. We also thank Gina Van Damme and Marlies De Coninck for their help in collecting the sputum samples and providing the clinical data of the study participants.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioflm.2024.100206.

References

[1] Elborn JS, Blasi F, Burgel PR, Peckham D. Role of inhaled antibiotics in the era of highly effective CFTR modulators. Eur Respir Rev 2023;32:220154. https://doi.org/10.1183/16000617.0154-2022.

[2] Cystic Fibrosis Foundation. Cystic Fibrosis Foundation Patient Registry. Annual data report. Bethesda: Maryland; 2022. 2023). Available online: https://www.cff.org/.

[3] Casaredi IG, Shaw M, Waters V, Seeto R, Blanchard AC, Ratjen F. Impact of antibiotic eradication therapy of *Pseudomonas aeruginosa* on long term lung function in cystic fibrosis. J Cyst Fibros 2023;22:98–102. https://doi.org/10.1016/j.jcf.2022.08.007.

[4] Pallenberg ST, Pust MM, Rosenboom I, Hansen G, Wiehlmann L, Dittrich AM, Tümmler B. Impact of elexacaftor/tezacaftor/ivacaftor therapy on the cystic fibrosis airway microbial metagenome. Microbiol Spectr 2022;10:e0145422. https://doi.org/10.1128/spectrum.01454-22.

[5] Beck MR, Hornick DB, Pena TA, Singh SB, Wright BA. Impact of elexacaftor/tezacaftor/ivacaftor on bacterial cultures from people with cystic fibrosis. Pediatr Pulmonol 2023;58:1569–73. https://doi.org/10.1002/ppul.26362.

[6] Hisert KB, Heltshe SL, Pope C, Jorth P, Wu X, Edwards RM, Radey M, Accurso FJ, Wolter DJ, Cooke G, Adam RJ, Carter S, Grogan B, Launspach JL, Donnelly SC, Gallagher CG, Bruce JE, Stoltz DA, Welsh MJ, Hoffman LR, McKone EF, Singh PK. Restoring cystic fibrosis transmembrane conductance regulator function reduces airway bacteria and inflammation in people with cystic fibrosis and chronic lung infections. Am J Respir Crit Care Med 2017;195:1617–28. https://doi.org/10.1164/rccm.201609-1954OC.

[7] Durfey SL, Pipavath S, Li A, Vo AT, Ratjen A, Carter S, Morgan SJ, Radey MC, Grogan B, Salipante SJ, Welsh MJ, Stoltz DA, Goss CH, McKone EF, Singh PK. Combining ivacaftor and intensive antibiotics achieves limited clearance of cystic fibrosis infections. mBio 2021;12:e0314821. https://doi.org/10.1128/mbio.03148-21.

[8] Nichols DP, Morgan SJ, Skalland M, Vo AT, Van Dalfsen JM, Singh SB, Ni W, Hoffman LR, McGeer K, Heltshe SL, Clancy JP, Rowe SM, Jorth P, Singh PK, PROMISE-Micro Study Group. Pharmacologic improvement of CFTR function rapidly decreases sputum pathogen density, but lung infections generally persist. J Clin Invest 2023;133:e167957. https://doi.org/10.1172/JCI167957.

[9] Armbruster CR, Hilliam YK, Zemke AC, Atteih S, Marshall CW, Moore J, Koirala J, Krainz L, Gaston JR, Lee SE, Cooper VS, Bomberger JM. Persistence and evolution of *Pseudomonas aeruginosa* following initiation of highly effective modulator therapy in cystic fibrosis. mBio 2024;2:e0051924. https://doi.org/10.1128/mbio.00519-24.

[10] Ledger EL, Smith DJ, Teh JJ, Wood ME, Whibley PE, Morrison M, Goldberg JB, Reid DW, Wells TJ. Impact of CFTR modulation on *Pseudomonas aeruginosa* infection in people with cystic fibrosis. J Infect Dis 2024;5:jiae051. https://doi.org/10.1093/infdis/jiae051.

[11] Malhotra S, Hayes D Jr, Wozniak DJ. Cystic fibrosis and *Pseudomonas aeruginosa*: the host-microbe interface. Clin Microbiol Rev 2019;32:e00138. https://doi.org/10.1128/CMR.00138-18. 18.

[12] Jennings LK, Dreifus JE, Reichhardt C, Storek KM, Secor PR, Wozniak DJ, Hisert KB, Parsek MR. *Pseudomonas aeruginosa* aggregates in cystic fibrosis sputum produce exopolysaccharides that likely impede current therapies. Cell Rep 2021;34:108782. https://doi.org/10.1016/j.celrep.2021.108782.

[13] Rossi E, La Rosa R, Bartell JA, Marvig RL, Haagensen JAJ, Sommer LM, Molin S, Johansen HK. *Pseudomonas aeruginosa* adaptation and evolution in patients with cystic fibrosis. Nat Rev Microbiol 2021;19:331–42. https://doi.org/10.1038/s41579-020-00477-5.

[14] Man WH, de Steenhuijsen Piters WA, Bogaert D. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol 2017;15:259–70. https://doi.org/10.1038/nrmicro.2017.14.

[15] Cuthbertson L, Walker AW, Oliver AE, Rogers GB, Rivett DW, Hampton TH, Ashare A, Elborn JS, De Soyza A, Carroll MP, Hoffman LR, Lanyon C, Moskowitz SM, O'Toole GA, Parkhill J, Planet PJ, Teneback CC, Tunney MM, Zuckerman JB, Bruce KD, van der Gast CJ. Lung function and microbiota diversity in cystic fibrosis. Microbiome 2020;8:45. https://doi.org/10.1186/s40168-020-00810-3.

[16] Armbruster CR, Coenye T, Touqui L, Bomberger JM. Interplay between host-microbe and microbe-microbe interactions in cystic fibrosis. J Cyst Fibros 2020;19:S47–53. https://doi.org/10.1016/j.jcf.2019.10.015.

[17] Welp AL, Bomberger JM. Bacterial community interactions during chronic respiratory disease. Front Cell Infect Microbiol 2020;10:213. https://doi.org/10.3389/fcimb.2020.00213.

[18] Thornton CS, Caverly LJ, LiPuma JJ. Coming up for air: the role of anaerobes in cystic fibrosis. Ann Am Thorac Soc 2022;19:713–6. https://doi.org/10.1513/AnnalsATS.202110-1142PS.

[19] O'Brien S, Fothergill JL. The role of multispecies social interactions in shaping *Pseudomonas aeruginosa* pathogenicity in the cystic fibrosis lung. FEMS Microbiol Lett 2017;364:fnx128. https://doi.org/10.1093/femsle/fnx128.

[20] Rezzoagli C, Granato ET, Kümmerli R. Harnessing bacterial interactions to manage infections: a review on the opportunistic pathogen *Pseudomonas aeruginosa* as a case example. J Med Microbiol 2020;69:147–61. https://doi.org/10.1099/jmm.0.001134.

[21] Cugini C, Morales DK, Hogan DA. *Candida albicans*-produced farnesol stimulates *Pseudomonas* quinolone signal production in LasR-defective *Pseudomonas aeruginosa* strains. Microbiology (Read) 2010;156:3096–107. https://doi.org/10.1099/mic.0.037911-0.

[22] Twomey KB, O'Connell OJ, McCarthy Y, Dow JM, O'Toole GA, Plant BJ, Ryan RP. Bacterial cis-2-unsaturated fatty acids found in the cystic fibrosis airway modulate virulence and persistence of *Pseudomonas aeruginosa*. ISME J 2012;6:939–50. https://doi.org/10.1038/ismej.2011.167.

[23] Korgaonkar A, Trivedi U, Rumbaugh KP, Whiteley M. Community surveillance enhances *Pseudomonas aeruginosa* virulence during polymicrobial infection. Proc Natl Acad Sci U S A 2013;110:1059–64. https://doi.org/10.1073/pnas.1214550110.

[24] Pompilio A, Crocetta V, De Nicola S, Verginelli F, Fiscarelli E, Di Bonaventura G. Cooperative pathogenicity in cystic fibrosis: *Stenotrophomonas maltophilia* modulates *Pseudomonas aeruginosa* virulence in mixed biofilm. Front Microbiol 2015;6:951. https://doi.org/10.3389/fmicb.2015.00951.

[25] Reece E, Doyle S, Greally P, Renwick J, McClean S. *Aspergillus fumigatus* inhibits *Pseudomonas aeruginosa* in co-culture: implications of a mutually antagonistic relationship on virulence and inflammation in the CF Airway. Front Microbiol 2018;9:1205. https://doi.org/10.3389/fmicb.2018.01205.

[26] Vandeplassche E, Tavernier S, Coenye T, Crabbé A. Influence of the lung microbiome on antibiotic susceptibility of cystic fibrosis pathogens. Eur Respir Rev 2019;28:190041. https://doi.org/10.1183/16000617.0041-2019.

[27] Reece E, Bettio PHA, Renwick J. Polymicrobial interactions in the cystic fibrosis airway microbiome impact the antimicrobial susceptibility of *Pseudomonas aeruginosa*. Antibiotics (Basel) 2021;10:827. https://doi.org/10.3390/antibiotics10070827.

[28] Scott JE, O'Toole GA. The yin and yang of *Streptococcus* lung infections in cystic fibrosis: a model for studying polymicrobial interactions. J Bacteriol 2019;201:e00115–9. https://doi.org/10.1128/JB.00115-19.

[29] Muhlebach MS, Hatch JE, Einarsson GG, McGrath SJ, Gilipin DF, Lavelle G, Mirkovic B, Murray MA, McNally P, Gotman N, Davis Thomas S, Wolfgang MC, Gilligan PH, McElvaney NG, Elborn JS, Boucher RC, Tunney MM. Anaerobic bacteria cultured from cystic fibrosis airways correlate to milder disease: a multisite study. Eur Respir J 2018;52:1800242. https://doi.org/10.1183/13993003.00242-2018.

[30] Lamoureux C, Guilloux CA, Beauruelle C, Gouriou S, Ramel S, Dirou A, Le Bihan J, Revert K, Ropars T, Lagrafeuille R, Vallet S, Le Berre R, Nowak E, Héry-Arnaud G. An observational study of anaerobic bacteria in cystic fibrosis lung using culture dependant and independent approaches. Sci Rep 2021;11:6845. https://doi.org/10.1038/s41598-021-85592-w.

[31] Tunney MM, Field TR, Moriarty TF, Patrick S, Doering G, Muhlebach MS, Wolfgang MC, Boucher R, Gilpin DF, McDowell A, Elborn JS. Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. Am J Respir Crit Care Med 2008;177:995–1001. https://doi.org/10.1164/rccm.200708-1151OC.

[32] Sibley CD, Grinwis ME, Field TR, Eshaghurshan CS, Faria MM, Dowd SE, Parkins MD, Rabin HR, Surette MG. Culture enriched molecular profiling of the cystic fibrosis airway microbiome. PLoS One 2011;6:e22702. https://doi.org/10.1371/journal.pone.0022702.
[33] Caverly LJ, LiPuma JJ. Good cop, bad cop: anaerobes in cystic fibrosis airways. Eur Respir J 2018;52:1801146. https://doi.org/10.1183/13993003.01146-2018.
[34] Thornton CS, Surette MG. Potential contributions of anaerobes in cystic fibrosis airways. J Clin Microbiol 2021;59:e01813. https://doi.org/10.1128/JCM.01813-19. 19.
[35] Flynn JM, Niccum D, Dunitz JM, Hunter RC. Evidence and role for bacterial mucin degradation in cystic fibrosis airway disease. PLoS Pathog 2016;12:e1005846. https://doi.org/10.1371/journal.ppat.1005846.
[36] Sherrard LJ, McGrath SJ, McIlreavey L, Hatch J, Wolfgang MC, Muhlebach MS, Gilpin DF, Elborn JS, Tunney MM. Production of extended-spectrum β-lactamases and the potential indirect pathogenic role of *Prevotella* isolates from the cystic fibrosis respiratory microbiota. Int J Antimicrob Agents 2016;47:140–5. https://doi.org/10.1016/j.ijantimicag.2015.12.004.
[37] Kesthely CA, Rogers RR, El Hafi B, Jean-Pierre F, O'Toole GA. Transcriptional profiling and genetic analysis of a cystic fibrosis airway-relevant model shows asymmetric responses to growth in a polymicrobial community. Microbiol Spectr 2023;11:e0220123. https://doi.org/10.1128/spectrum.02201-23.
[38] Bertelsen A, Elborn JS, Schock BC. Microbial interaction: *Prevotella* spp. reduce *P. aeruginosa* induced inflammation in cystic fibrosis bronchial epithelial cells. J Cyst Fibros 2021;20:682–91. https://doi.org/10.1016/j.jcf.2021.04.012.
[39] Goeteyn E, Grassi L, Van den Bossche S, Rigauts C, Vande Weygaerde Y, Van Braeckel E, Maes T, Bracke KR, Crabbé A. Commensal bacteria of the lung microbiota synergistically inhibit inflammation in a three-dimensional epithelial cell model. Front Immunol 2023;14:1176044. https://doi.org/10.3389/fimmu.2023.1176044.
[40] Zemanick ET, Wagner BD, Robertson CE, Stevens MJ, Szefler SJ, Accurso FJ, Sagel SD, Harris JK. Assessment of airway microbiota and inflammation in cystic fibrosis using multiple sampling methods. Ann Am Thorac Soc 2015;12:221–9. https://doi.org/10.1513/AnnalsATS.201407-310OC.
[41] Zemanick ET, Wagner BD, Robertson CE, Ahrens RC, Chmiel JF, Clancy JP, Gibson RL, Harris WT, Kurland G, Laguna TA, McColley SA, McCoy K, Retsch-Bogart G, Sobush KT, Zeitlin PL, Stevens MJ, Accurso FJ, Sagel SD, Harris JK. Airway microbiota across age and disease spectrum in cystic fibrosis. Eur Respir J 2017;50:1700832. https://doi.org/10.1183/13993003.00832-2017.
[42] Einarsson GG, Ronan NJ, Mooney D, McGettigan C, Mullane D, NiChroinin M, Shanahan F, Murphy DM, McCarthy M, McCarthy Y, Eustace JA, Gilpin DF, Elborn JS, Plant BJ, Tunney MM. Extended-culture and culture-independent molecular analysis of the airway microbiota in cystic fibrosis following CFTR modulation with ivacaftor. J Cyst Fibros 2021;20:747–53. https://doi.org/10.1016/j.jcf.2020.12.023.
[43] Zhao CY, Hao Y, Wang Y, Varga JJ, Stecenko AA, Goldberg JB, Brown SP. Microbiome data enhances predictive models of lung function in people with cystic fibrosis. J Infect Dis 2021;223:S246–56. https://doi.org/10.1093/infdis/jiaa655.
[44] O'Neill K, Bradley JM, Johnston E, McGrath S, McIlreavey L, Rowan S, Reid A, Bradbury I, Einarsson G, Elborn JS, Tunney MM. Reduced bacterial colony count of anaerobic bacteria is associated with a worsening in lung clearance index and inflammation in cystic fibrosis. PLoS One 2015;10:e0126980. https://doi.org/10.1371/journal.pone.0126980.
[45] De Soyza A, Hall AJ, Mahenthiralingam E, Drevinek P, Kaca W, Drulis-Kawa Z, Stoitsova SR, Toth V, Coenye T, Zlosnik JE, Burns JL, Sá-Correia I, De Vos D, Pirnay JP, Kidd TJ, Reid D, Manos J, Klockgether J, Wiehlmann L, Tümmler B, McClean S, Winstanley C. Developing an international *Pseudomonas aeruginosa* reference panel. MicrobiologyOpen 2013;2:1010–23. https://doi.org/10.1002/mbo3.141.
[46] Grace A, Sahu R, Owen DR, Dennis VA. *Pseudomonas aeruginosa* reference strains PAO1 and PA14: a genomic, phenotypic, and therapeutic review. Front Microbiol 2022;13:1023523. https://doi.org/10.3389/fmicb.2022.1023523.
[47] Bragonzi A, Wiehlmann L, Klockgether J, Cramer N, Worlitzsch D, Döring G, Tümmler B. Sequence diversity of the mucABD locus in *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. Microbiology (Read) 2006;152:3261–9. https://doi.org/10.1099/mic.0.29175-0.
[48] Vandeplassche E, Sass A, Lemarcq A, Dandekar AA, Coenye T, Crabbé A. *In vitro* evolution of *Pseudomonas aeruginosa* AA2 biofilms in the presence of cystic fibrosis lung microbiome members. Sci Rep 2019;9:12859. https://doi.org/10.1038/s41598-019-49371-y.
[49] Mulcahy LR, Burns JL, Lory S, Lewis K. Emergence of *Pseudomonas aeruginosa* strains producing high levels of persister cells in patients with cystic fibrosis. J Bacteriol 2010;192:6191–9. https://doi.org/10.1128/JB.01651-09.
[50] Feltner JB, Wolter DJ, Pope CE, Groleau MC, Smalley NE, Greenberg EP, Mayer-Hamblett N, Burns J, Déziel E, Hoffman LR, Dandekar AA. LasR variant cystic fibrosis isolates reveal an adaptable quorum-sensing hierarchy in *Pseudomonas aeruginosa*. mBio 2016;7:e01513. https://doi.org/10.1128/mBio.01513-16. 16.
[51] Leitão JH, Alvim T, Sá-Correia I. Ribotyping of *Pseudomonas aeruginosa* isolates from patients and water springs and genome fingerprinting of variants concerning mucoidy. FEMS Immunol Med Microbiol 1996;13:287–92. https://doi.org/10.1111/j.1574-695X.1996.tb00253.x.
[52] Pirnay JP, Bilocq F, Pot B, Cornelis P, Zizi M, Van Eldere J, Deschaght P, Vaneechoutte M, Jennes S, Pitt T, De Vos D. *Pseudomonas aeruginosa* population structure revisited. PLoS One 2009;4:e7740. https://doi.org/10.1371/journal.pone.0007740.
[53] Mathee K, Narasimhan G, Valdes C, Qiu X, Matewish JM, Koehrsen M, Rokas A, Yandava CN, Engels R, Zeng E, Olavarietta R, Doud M, Smith RS, Montgomery P, White JR, Godfrey PA, Kodira C, Birren B, Galagan JE, Lory S. Dynamics of *Pseudomonas aeruginosa* genome evolution. Proc Natl Acad Sci U S A 2008;105:3100–5. https://doi.org/10.1073/pnas.0711982105.
[54] Field TR, Sibley CD, Parkins MD, Rabin HR, Surette MG. The genus *Prevotella* in cystic fibrosis airways. Anaerobe 2010;16:337–44. https://doi.org/10.1016/j.anaerobe.2010.04.002.
[55] Whelan FJ, Waddell B, Syed SA, Shekarriz S, Rabin HR, Parkins MD, Surette MG. Culture-enriched metagenomic sequencing enables in-depth profiling of the cystic fibrosis lung microbiota. Nat Microbiol 2020;5:379–90. https://doi.org/10.1038/s41564-019-0643-y.
[56] Gürsoy M, Harju I, Matomäki J, Bryk A, Könönen E. Performance of MALDI-TOF MS for identification of oral *Prevotella* species. Anaerobe 2017;47:89–93. https://doi.org/10.1016/j.anaerobe.2017.04.008.
[57] Cobo F, Pérez-Carrasco V, Martín-Hita L, García-Salcedo JA, Navarro-Marí JM. Comparative evaluation of MALDI-TOF MS and 16S rRNA gene sequencing for the identification of clinically relevant anaerobic bacteria: critical evaluation of discrepant results. Anaerobe 2023;82:102754. https://doi.org/10.1016/j.anaerobe.2023.102754.
[58] Alcalá L, Marín M, Ruiz A, Quiroga L, Zamora-Cintas M, Fernández-Chico MA, Muñoz P, Rodríguez-Sánchez B. Identifying anaerobic bacteria using MALDI-TOF mass spectrometry: a four-year experience. Front Cell Infect Microbiol 2021;11:521014. https://doi.org/10.3389/fcimb.2021.521014.
[59] Christiaen SE, Matthijs N, Zhang XH, Nelis HJ, Bossier P, Coenye T. Bacteria that inhibit quorum sensing decrease biofilm formation and virulence in *Pseudomonas aeruginosa* PAO1. Pathog Dis 2014;70:271–9. https://doi.org/10.1111/2049-632X.12124.
[60] Tjørve KMC, Tjørve E. The use of Gompertz models in growth analyses, and new Gompertz-model approach: an addition to the Unified-Richards family. PLoS One 2017;12:e0178691. https://doi.org/10.1371/journal.pone.0178691.
[61] Peeters E, Nelis HJ, Coenye T. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. J Microbiol Methods 2008;72:157–65. https://doi.org/10.1016/j.mimet.2007.11.010.
[62] Vandeplassche E, Sass A, Ostyn L, Burmølle M, Kragh KN, Bjarnsholt T, Coenye T, Crabbé A. Antibiotic susceptibility of cystic fibrosis lung microbiome members in a multispecies biofilm. Biofilms 2020;2:100031. https://doi.org/10.1016/j.bioflm.2020.100031.
[63] Zhang Y, Sass A, Van Acker H, Wille J, Verhasselt B, Van Nieuwerburgh F, Kaever V, Crabbé A, Coenye T. Coumarin reduces virulence and biofilm formation in *Pseudomonas aeruginosa* by affecting quorum sensing, type III secretion and c-di-GMP levels. Front Microbiol 2018;9:1952. https://doi.org/10.3389/fmicb.2018.01952.
[64] Webb KA, Olagoke O, Baird T, Neill J, Pham A, Wells TJ, Ramsay KA, Bell SC, Sarovich DS, Price EP. Genomic diversity and antimicrobial resistance of *Prevotella* species isolated from chronic lung disease airways. Microb Genom 2022;8:000754. https://doi.org/10.1099/mgen.0.000754.
[65] Burmølle M, Thomsen TR, Fazli M, Dige I, Christensen L, Homøe P, Tvede M, Nyvad B, Tolker-Nielsen T, Givskov M, Moser C, Kirketerp-Møller K, Johansen HK, Høiby N, Jensen PØ, Sørensen SJ, Bjarnsholt T. Biofilms in chronic infections - a matter of opportunity - monospecies biofilms in multispecies infections. FEMS Immunol Med Microbiol 2010;59:324–36. https://doi.org/10.1111/j.1574-695X.2010.00714.x.
[66] Rudkjøbing VB, Thomsen TR, Alhede M, Kragh KN, Nielsen PH, Johansen UR, Givskov M, Høiby N, Bjarnsholt T. The microorganisms in chronically infected end-stage and non-end-stage cystic fibrosis patients. FEMS Immunol Med Microbiol 2012;65:236–44. https://doi.org/10.1111/j.1574-695X.2011.00925.x.
[67] Cullen L, Weiser R, Olszak T, Maldonado RF, Moreira AS, Slachmuylders L, Brackman G, Paunova-Krasteva TS, Zarnowiec P, Czerwonka G, Reilly J, Drevinek P, Kaca W, Melter O, De Soyza A, Perry A, Winstanley C, Stoitsova SR, Lavigne R, Mahenthiralingam E, Sá-Correia I, Coenye T, Drulis-Kawa Z, Augustyniak D, Valvano MA, McClean S. Phenotypic characterization of an international *Pseudomonas aeruginosa* reference panel: strains of cystic fibrosis (CF) origin show less in vivo virulence than non-CF strains. Microbiology (Read) 2015;161:1961–77. https://doi.org/10.1099/mic.0.000155.
[68] Caldwell CC, Chen Y, Goetzmann HS, Hao Y, Borchers MT, Hassett DJ, Young LR, Mavrodi D, Thomashow L, Lau GW. *Pseudomonas aeruginosa* exotoxin pyocyanin causes cystic fibrosis airway pathogenesis. Am J Pathol 2009;175:2473–88. https://doi.org/10.2353/ajpath.2009.090166.
[69] Sandri A, Ortombina A, Boschi F, Cremonini E, Boaretti M, Sorio C, Melotti P, Bergamini G, Lleo M. Inhibition of *Pseudomonas aeruginosa* secreted virulence factors reduces lung inflammation in CF mice. Virulence 2018;9:1008–18. https://doi.org/10.1080/21505594.2018.1489198.
[70] Jansen HJ, Grenier D, Van der Hoeven JS. Characterization of immunoglobulin G-degrading proteases of *Prevotella intermedia* and *Prevotella nigrescens*. Oral Microbiol Immunol 1995;10:138–45. https://doi.org/10.1111/j.1399-302x.1995.tb00134.x.
[71] Potempa M, Potempa J, Kantyka T, Nguyen KA, Wawrzonek K, Manandhar SP, Popadiak K, Riesbeck K, Eick S, Blom AM. Interpain A, a cysteine proteinase from *Prevotella intermedia*, inhibits complement by degrading complement factor C3. PLoS Pathog 2009;5:e1000316. https://doi.org/10.1371/annotation/e82f810e-738a-47e5-9295-5a0cc9a0dc6c. 10.1371/journal.ppat.1000316. Erratum in: PLoS Pathog (2009). 5(3.
[72] Ulrich M, Beer I, Braitmaier P, Dierkes M, Kummer F, Krismer B, Schumacher U, Gräpler-Mainka U, Riethmüller J, Jensen PØ, Bjarnsholt T, Høiby N, Bellon G, Döring G. Relative contribution of *Prevotella intermedia* and *Pseudomonas aeruginosa* to lung pathology in airways of patients with cystic fibrosis. Thorax 2010;65:978–84. https://doi.org/10.1136/thx.2010.137745.

[73] Thornton CS, Acosta N, Surette MG, Parkins MD. Exploring the cystic fibrosis lung microbiome: making the most of a sticky situation. J Pediatric Infect Dis Soc 2022; 11:S13–22. https://doi.org/10.1093/jpids/piac036.

[74] Pustelny C, Komor U, Pawar V, Lorenz A, Bielecka A, Moter A, Gocht B, Eckweiler D, Müsken M, Grothe C, Lünsdorf H, Weiss S, Häussler S. Contribution of *Veillonella parvula* to *Pseudomonas aeruginosa*-mediated pathogenicity in a murine tumor model system. Infect Immun 2015;83:417–29. https://doi.org/10.1128/IAI.02234-14.

[75] Venkataraman A, Rosenbaum MA, Werner JJ, Winans SC, Angenent LT. Metabolite transfer with the fermentation product 2,3-butanediol enhances virulence by *Pseudomonas aeruginosa*. ISME J 2014;8:1210–20. https://doi.org/10.1038/ismej.2013.232.

[76] Nguyen M, Sharma A, Wu W, Gomi R, Sung B, Hospodsky D, Angenent LT, Worgall S. The fermentation product 2,3-butanediol alters *P. aeruginosa* clearance, cytokine response and the lung microbiome. ISME J 2016;10:2978–83. https://doi.org/10.1038/ismej.2016.76.

[77] Phan J, Gallagher T, Oliver A, England WE, Whiteson K. Fermentation products in the cystic fibrosis airways induce aggregation and dormancy-associated expression profiles in a CF clinical isolate of *Pseudomonas aeruginosa*. FEMS Microbiol Lett 2018;365:fny082. https://doi.org/10.1093/femsle/fny082.

[78] Stoner SN, Baty JJ, Novak L, Scoffield JA. Commensal colonization reduces *Pseudomonas aeruginosa* burden and subsequent airway damage. Front Cell Infect Microbiol 2023;13:1144157. https://doi.org/10.3389/fcimb.2023.1144157.

[79] Morris AJ, Yau YCW, Park S, Eisha S, McDonald N, Parsek MR, Howell PL, Hoffman LR, Nguyen D, DiGiandomenico A, Rooney AM, Coburn B, Grana-Miraglia L, Wang P, Guttman DS, Wozniak DJ, Waters VJ. *Pseudomonas aeruginosa* aggregation and Psl expression in sputum is associated with antibiotic eradication failure in children with cystic fibrosis. Sci Rep 2022;12:21444. https://doi.org/10.1038/s41598-022-25889-6.

[80] Chappell TC, Nair NU. Engineered lactobacilli display anti-biofilm and growth suppressing activities against *Pseudomonas aeruginosa*. NPJ Biofilms Microbiomes 2020;6:48. https://doi.org/10.1038/s41522-020-00156-6.

[81] Batoni G, Catelli E, Kaya E, Pompilio A, Bianchi M, Ghelardi E, Di Bonaventura G, Esin S, Maisetta G. Antibacterial and antibiofilm effects of lactobacilli strains against clinical isolates of *Pseudomonas aeruginosa* under conditions relevant to cystic fibrosis. Antibiotics (Basel) 2023;12:1158. https://doi.org/10.3390/antibiotics12071158.

[82] Trizna E, Bogachev MI, Kayumov A. Degrading of the *Pseudomonas aeruginosa* biofilm by extracellular levanase SacC from *Bacillus subtilis*. BioNanoScience 2019; 9:48–52. https://doi.org/10.1007/s12668-018-0581-9.

[83] Daboor SM, Rohde JR, Cheng Z. Disruption of the extracellular polymeric network of *Pseudomonas aeruginosa* biofilms by alginate lyase enhances pathogen eradication by antibiotics. J Cyst Fibros 2021;20:264–70. https://doi.org/10.1016/j.jcf.2020.04.006.

[84] LaFayette SL, Houle D, Beaudoin T, Wojewodka G, Radzioch D, Hoffman LR, Burns JL, Dandekar AA, Smalley NE, Chandler JR, Zlosnik JE, Speert DP, Bernier J, Matouk E, Brochiero E, Rousseau S, Nguyen D. Cystic fibrosis-adapted *Pseudomonas aeruginosa* quorum sensing lasR mutants cause hyperinflammatory responses. Sci Adv 2015;1:e1500199. https://doi.org/10.1126/sciadv.1500199.

[85] Hennemann LC, LaFayette SL, Malet JK, Bortolotti P, Yang T, McKay GA, Houle D, Radzioch D, Rousseau S, Nguyen D. LasR-deficient *Pseudomonas aeruginosa* variants increase airway epithelial mICAM-1 expression and enhance neutrophilic lung inflammation. PLoS Pathog 2021;17:e1009375. https://doi.org/10.1371/journal.ppat.1009375.

[86] Jean-Pierre F, Hampton TH, Schultz D, Hogan DA, Groleau MC, Déziel E, O'Toole GA. Community composition shapes microbial-specific phenotypes in a cystic fibrosis polymicrobial model system. Elife 2023;12:e81604. https://doi.org/10.7554/eLife.81604.

[87] Duan K, Dammel C, Stein J, Rabin H, Surette MG. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. Mol Microbiol 2003;50:1477–91. https://doi.org/10.1046/j.1365-2958.2003.03803.x.

[88] Whiley RA, Sheikh NP, Mushtaq N, Hagi-Pavli E, Personne Y, Javaid D, Waite RD. Differential potentiation of the virulence of the *Pseudomonas aeruginosa* cystic fibrosis liverpool epidemic strain by oral commensal Streptococci. J Infect Dis 2014;209:769–80. https://doi.org/10.1093/infdis/jit568.

[89] Waite RD, Qureshi MR, Whiley RA. Modulation of behaviour and virulence of a high alginate expressing *Pseudomonas aeruginosa* strain from cystic fibrosis by oral commensal bacterium *Streptococcus anginosus*. PLoS One 2017;12:e0173741. https://doi.org/10.1371/journal.pone.0173741. Erratum in: PLoS One (2017). 12: e0176577.

[90] Sibley CD, Duan K, Fischer C, Parkins MD, Storey DG, Rabin HR, Surette MG. Discerning the complexity of community interactions using a *Drosophila* model of polymicrobial infections. PLoS Pathog 2008;4:e1000184. https://doi.org/10.1371/journal.ppat.1000184.

[91] Tett A, Pasolli E, Masetti G, Ercolini D, Segata N. Prevotella diversity, niches and interactions with the human host. Nat Rev Microbiol 2021;19:585–99. https://doi.org/10.1038/s41579-021-00559-y.