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Microbiology, Genomics, and Clinical Significance of the *Pseudomonas fluorescens* Species Complex, an Unappreciated Colonizer of Humans

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SUMMARY

Pseudomonas fluorescens is not generally considered a bacterial pathogen in humans; however, multiple culture-based and culture-independent studies have identified it at low levels in the indigenous microbiota of various body sites. With recent advances in comparative genomics, many isolates originally identified as the "species" *P. fluorescens* are now being reclassified as novel *Pseudomonas* species within the *P. fluorescens* "species complex." Although most widely studied for its role in the soil and the rhizosphere, *P. fluorescens* possesses a number of functional traits that provide it with the capability to grow and thrive in mammalian hosts. While significantly less virulent than *P. aeruginosa*, *P. fluorescens* can cause bacteremia in humans, with most reported cases being attributable either to transfusion of contaminated blood products or to use of contaminated equipment associated with intravenous infusions. Although not suspected of being an etiologic agent of pulmonary disease, there are a number of reports identifying it in respiratory samples. There is also an intriguing association between *P. fluorescens* and human disease, in that approximately 50% of Crohn's disease patients develop serum antibodies to *P. fluorescens*. Altogether, these reports are beginning to highlight a far more common, intriguing, and potentially complex association between humans and *P. fluorescens* during health and disease.

INTRODUCTION

Over the past 15 years, the application of culture-independent methods for microbial identification has revealed a previously unappreciated complexity within human-microbe interactions. One interesting feature is that a number of these studies have identified the bacterium *Pseudomonas fluorescens* as a low-abundance member of the indigenous microbiota of various body sites, including the mouth, stomach, and lungs (1–5). *P. fluorescens* has generally been considered nonpathogenic for humans, an assessment dating back to its earliest descriptions, by A. Baader and C. Garre, in *Über Antagonisten unter den Bacterien* (1887) (6):

> The bacillus [*P. fluorescens*] itself is not pathogenic. A culture applied to animals subcutaneously or injected into the peritoneum does not elicit a reaction. Even when introduced many times into fresh wounds it does not irritate healing by primary intention. Also, ingestion of cultures caused no harm to my stomach or intestines.

However, while far less virulent than *P. aeruginosa*, *P. fluorescens* can cause acute infections (opportunistic) in humans and has been reported in clinical samples from the mouth, stomach, and lungs (Table 1). The most common site of *P. fluorescens* infection is the bloodstream. Most reported cases have been iatrogenic, with bacteremia attributable either to transfusion of contaminated blood products (7–12) or to use of contaminated equipment associated with intravenous infusions (13–17). While

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doi:10.1128/CMR.00044-14

TABLE 1 Reported *P. fluorescens* infections

Organ or tissue	No. of reported cases[a]	Reference(s)
Blood	110	8–17, 183–189
Bone	2	213, 214
Cerebrospinal fluid	1	215
Eye	3	216–218
Lung	3	195–198
Sinus	3	219
Skin/wound	5	190, 191, 194
Urinary tract	5	192–194
Uterus	1	220

[a] Total number of cases reported in the medical literature. MEDLINE searches were performed with the search term "*Pseudomonas fluorescens*" and filtered for human studies, with no date or language restrictions. All abstracts were read and reviewed by us, and relevant references were read in their entirety.

TABLE 2 *P. fluorescens* isolates cultured over an 11-year period by the University of Michigan Hospital Microbiology Lab[a]

Parameter[b]	% of isolates
Culture method	
Cultured using routine laboratory protocols	59.50
Cultured using modified CF protocols	40.10
Sample type	
Sputum samples	53.70
Throat swabs	21.10
Bronchoscopically obtained samples (BAL fluids or brushings)	13.20
Other (tracheal aspirates, sinus aspirates)	12.00
Underlying disease/cause	
CF	38.80
Other chronic airway disease (COPD, asthma, non-CF bronchiectasis)	16.10
Lung transplantation	7.40
Acute pneumonia (in chronically immunosuppressed patient or hospital acquired)	9.90
Acute pneumonia (not in chronically immunosuppressed patient or hospital acquired)	1.60
Other (chronic tracheostomy, sinusitis, acute respiratory distress syndrome, bone marrow transplantation)	26.20
Cocultured bacteria	
"Oral flora" species	85.10
Pseudomonas aeruginosa	25.60
Staphylococcus aureus	15.70
Stenotrophomonas maltophilia	11.60

[a] The data show a breakdown of 242 *P. fluorescens* isolates cultured between 1 January 2002 and 13 December 2012 (3).
[b] CF, cystic fibrosis; BAL, bronchoalveolar lavage.

not suspected of being an etiologic agent of pulmonary disease, we recently reported that *P. fluorescens* is routinely cultured at a low frequency from clinically indicated respiratory samples (3) (Table 2). Perhaps the most intriguing "association" between *P. fluorescens* and human disease is that approximately 50% of Crohn's disease patients develop serum antibodies to the I2 antigen encoded by *P. fluorescens*, and in some studies, this seroreactivity has correlated with the success of therapies aimed at the microbiome rather than the immune system (18–22). Altogether, these reports and others are beginning to highlight a far more common, and potentially complex, interaction between humans and *P. fluorescens* during health and disease.

The extremely versatile metabolic capabilities of *P. fluorescens* impart this bacterium with the ability to persist in a wide range of environments beyond mammalian hosts (Fig. 1), including soil, the rhizospheres and surfaces of plants, nonsterile pharmaceuticals, showerheads, and even indoor wall surfaces (23, 24). *P. fluorescens* has been studied most widely as an environmental microbe, most notably for its role in promoting plant health via a number of encoded antimicrobial mechanisms (25–38). However, *P. fluorescens* also possesses a number of functional traits that provide it with the capability to grow and thrive in mammalian hosts, including production of bioactive secondary metabolites (26–30, 33, 39–42), siderophores (43–45), and a type III secretion system (46–51), the ability to form biofilms (20, 52–56), and the plasticity of some strains to adapt to growth at higher temperature (53, 57–59).

With recent rapid advancements in taxonomy and comparative genomics, many *Pseudomonas* isolates originally identified as the "species" *P. fluorescens* are now being reclassified as novel *Pseudomonas* species within the *P. fluorescens* "species complex" (23, 60, 61). There are at least 52 species within this group (Fig. 2), and they share many phenotypic characteristics (Fig. 3). Since the taxonomic reclassifications within *P. fluorescens* are relatively new and ongoing, and beyond the scope of this review, we use the term "*P. fluorescens* species complex," or simply "*P. fluorescens*," in this review for studies on any isolates within this *Pseudomonas* species complex (Fig. 2).

PHENOTYPIC TRAITS AND CULTIVATION OF *P. FLUORESCENS*

The bacteria in the *P. fluorescens* species complex are Gram-negative, motile rods that are primarily aerobic, unable to ferment glucose, and chemoorganotrophic and grow at a pH between 4 and 8 (62) (Table 3 and Fig. 3). Isolates of *P. fluorescens* derived from nonmammalian samples have a permissive growth range of 4 to 32°C (62), while isolates from humans and other mammals have an elevated upper range extending to 37°C (53, 57–59). As of the end of 2013, there were 16 fully sequenced strains from the *P. fluorescens* species complex, and all but one originated from plant surfaces, roots, or the surrounding soil (Table 4). *P. fluorescens* can also be found in an antagonistic relationship with eukaryotic microbes, including oomycetes and amoeba (35, 36, 48, 51, 59, 63–65), with the latter relationship potentially reflecting conserved mechanisms that are also used with macrophages, as has been hypothesized for other bacteria (66).

Like most members of the *Pseudomonas* genus, *P. fluorescens* species complex strains grow best in a rich, peptide-containing medium with a 0.1 to 1.0% (wt/vol) energy source (62). Examples of such basic media include nutrient broth/agar and tryptic soy broth/agar (62). Selective media that are deficient in iron allow for the detection of the natural fluorescence produced by these bacteria, which is enhanced due to increased production of fluorescent siderophores. King's A and B media (67), Pseudosel agar medium (BBL Microbiology Systems), and *Pseudomonas* agar F medium (Difco Laboratories, Detroit, MI) are all examples of pigment-enhancing media. These media also contain additional compounds, such as potassium, magnesium, and/or cetrimide, that further enable selective growth of *P. fluorescens* species com-

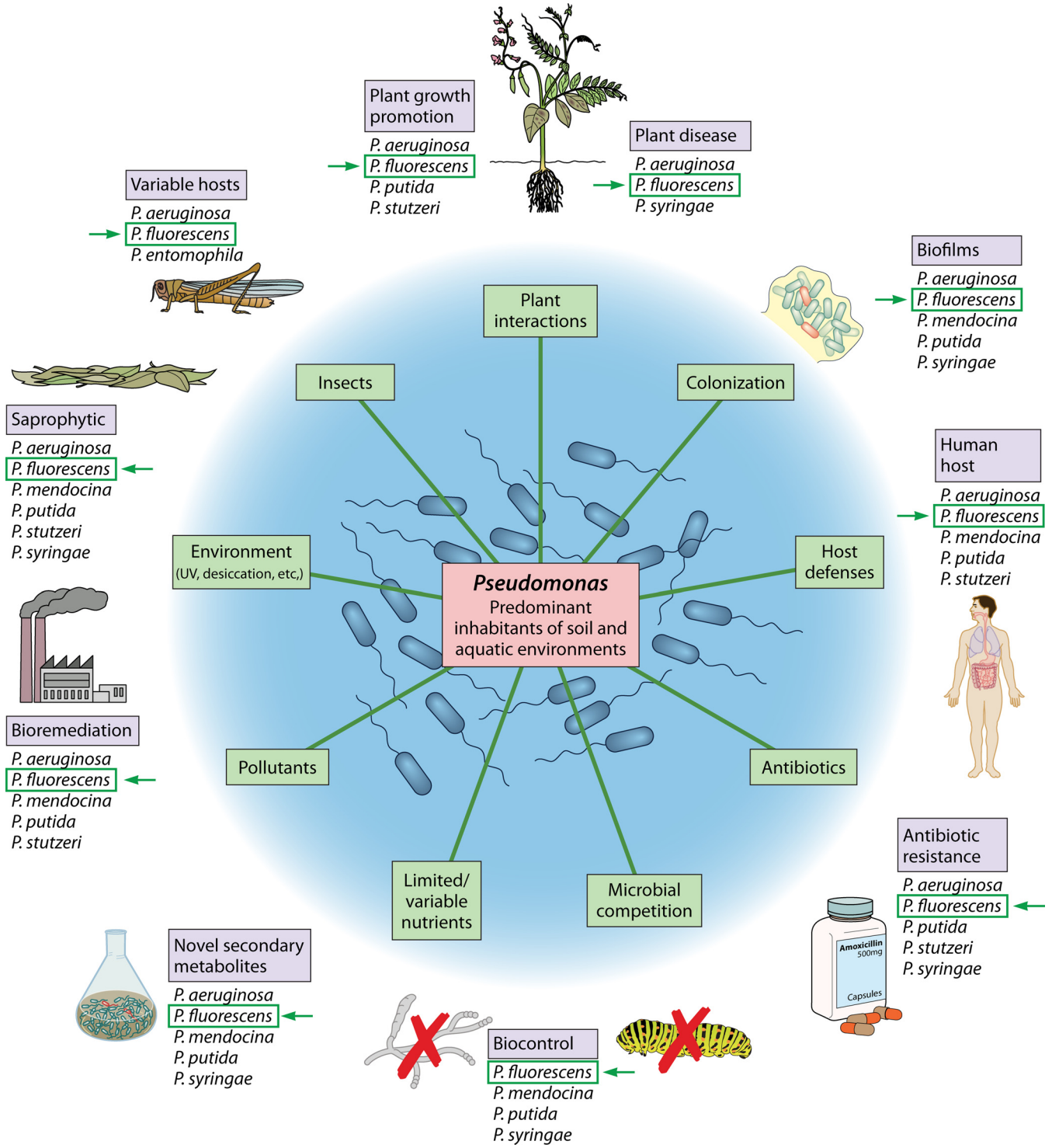

FIG 1 Functional range and environmental niches of the *Pseudomonas* genus, highlighting the broad distribution of the *P. fluorescens* species complex. Members of the *P. fluorescens* species complex are successful colonizers in a wide range of environments and habitats due to diverse functional abilities. (Reprinted from reference 208 with permission of John Wiley and Sons [copyright 2011 Federation of European Microbiological Societies].)

plex bacteria. Cetrimide in particular helps to inhibit the growth of non-*Pseudomonas* microbial flora and allows for adequate pigment production from *P. aeruginosa* (68). One of the difficulties in isolation of particular species of the *Pseudomonas* genus is that they share many of the same phenotypic traits and grow under the same cultivation conditions. However, it is possible to use pigment production, which varies by species group, to visibly distinguish isolates from different groups. The blue-green pigment pyo-

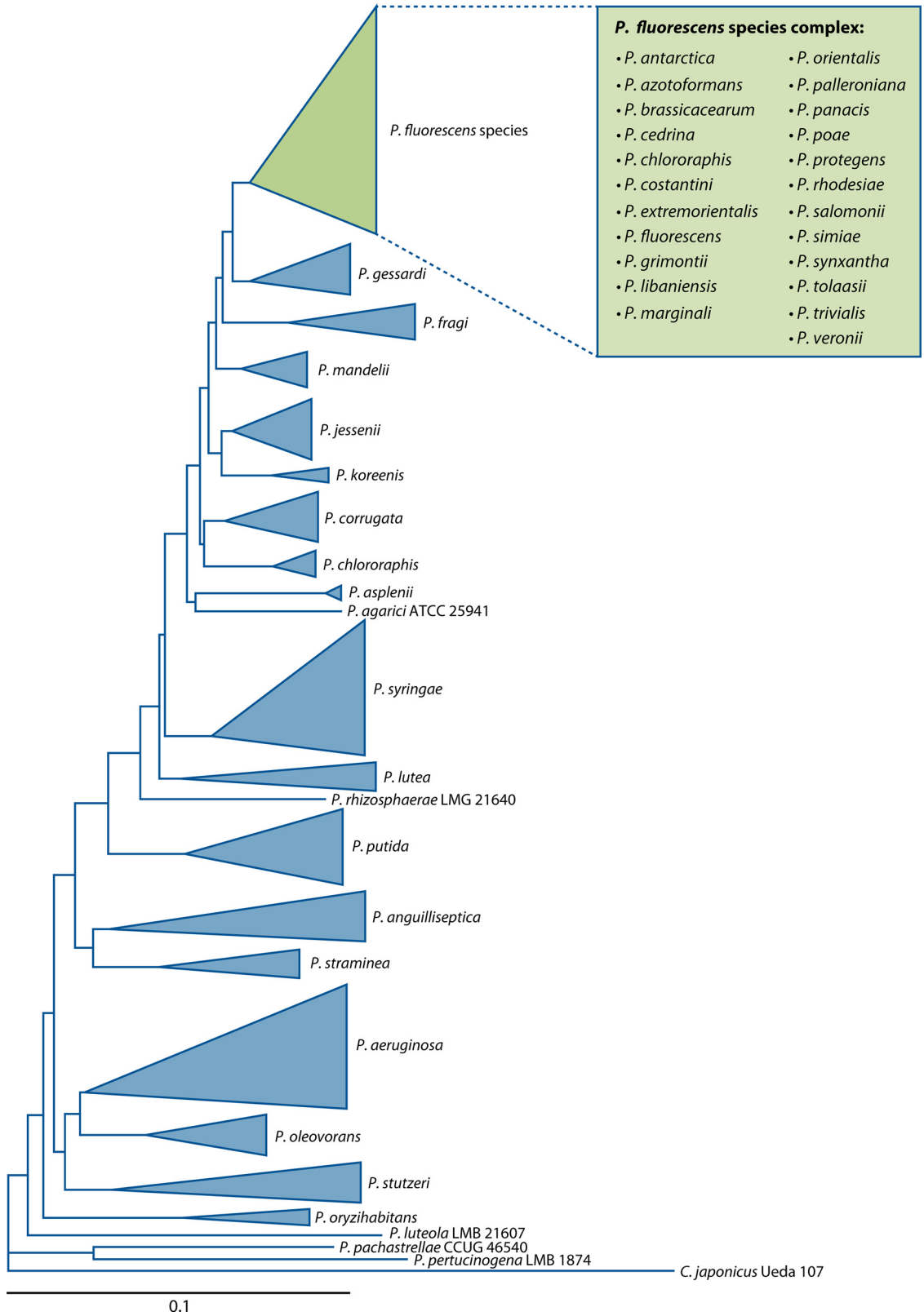

FIG 2 Species diversity within the *P. fluorescens* species complex. Mulet et al. generated a phylogenetic tree from 107 *Pseudomonas* type strains, based on concatenated analysis of the 16S rRNA, *gyrB*, *rpoB*, and *rpoD* genes, with *Cellvibrio japonicum* Ueda107 included as the outgroup (74). The bar indicates sequence divergence. (Reproduced from reference 74 with permission of John Wiley and Sons [copyright 2010 Society for Applied Microbiology and Blackwell Publishing Ltd.]. The names of the *Pseudomonas* species that have been included in the *P. fluorescens* species complex were added to the original figure.)

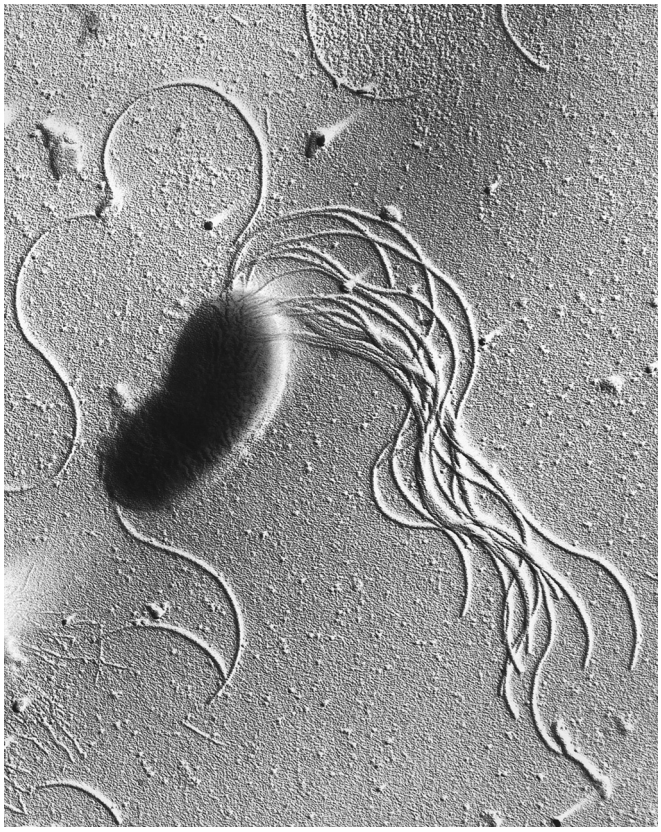

FIG 3 Scanning electron micrograph of *P. fluorescens*. (Photo reprinted with permission of Science Source.)

cyanin, readily produced by *P. aeruginosa* strains, is typically not produced by strains of the *P. fluorescens* species complex (62). Therefore, a mixed culture of *P. fluorescens* species complex bacteria and *P. aeruginosa* bacteria grown on cetrimide agar will produce blue fluorescent colonies of *P. aeruginosa* and nonblue fluorescent colonies of *P. fluorescens* complex bacteria.

Environmental isolates of *P. fluorescens* are readily cultivated in the laboratory by use of standard culturing techniques at a lower temperature range (5°C to 32°C), but in samples from higher temperatures or in clinical material, cultivation of *P. fluorescens* may be more difficult. *P. fluorescens* can be cultivated from environmental samples by using a simple medium with a carbon source and aerobic incubation for 24 to 48 h at 27°C to 32°C (62). However, incubation of environmental samples at temperatures of 35°C to 37°C can cause *P. fluorescens* to enter a viable-but-not-culturable (VBNC) state (69), complicating cultivation. During the VBNC state, bacteria are still metabolically active but are unable to undergo cellular division and replication (70). Bacteria in a VBNC state often will not grow when immediately transferred to standard culture conditions. *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica* serovar Enteritidis, and *Shigella dysenteriae* are all examples of bacteria that can enter into a VBNC state (71). *Vibrio* species also undergo a switch to a VBNC state that, similar to the case of *P. fluorescens*, is prompted by a switch in temperature (72). The VBNC state is hypothesized to be a survival strategy that allows bacteria to persist in harsh environments (73). The ability of *P. fluorescens* to become VBNC could explain the phenomenon in which *P. fluorescens* can be found more frequently in human lung metagenomic DNA than is reported by standard hospital culture methods (3). However, some isolates of *P. fluorescens* from human samples have adapted well to a higher permissive temperature range than that for isolates from environmental samples. For example, we have a collection of over 30 *P. fluorescens* strains from cystic fibrosis patients that grow well at 37°C. Another study reported a series of *P. fluorescens* isolates from surface abscess, septicemia, and respiratory or urinary tract infections that were able to grow at 37°C (57). All seven were also able to grow at 4°C, often considered the lower limit of the optimal temperature range of *Pseudomonas* spp. (62), suggesting that these strains did not shift their temperature range but, rather, the range expanded upwards.

GENOMICS

Taxonomy and Genomics of the *Pseudomonas* Genus and the *P. fluorescens* Species Complex

Of the many species within the *Pseudomonas* genus, the *P. fluorescens* species complex contains ~20% (74). As of January 2014, the List of Prokaryotic Names with Standing in Nomenclature (LPSN) recognized 211 species and 18 subspecies in the *Pseudomonas* genus (http://www.bacterio.net/pseudomonas.html). This reflects a 40% increase in newly defined *Pseudomonas* species compared to the number in 2006 (74). In the last few decades, isolates classified as *P. fluorescens* have undergone extensive renaming and reorganization, consistent with the high degree of genomic diversity within this species complex (75). Historically, any bacterium that was a Gram-negative, strictly aerobic, nonsporulating, motile bacillus was classified as belonging to the *Pseudomonas* genus (76). The name *Pseudomonas* derives from the Greek words for "false" (*pseudes*) and "single unit" (*monas*), so it

TABLE 3 Characteristics of *P. fluorescens* complex bacteria

Characteristic
Taxonomy
Bacteria, *Proteobacteria*, *Gammaproteobacteria*, *Pseudomonadales*, *Pseudomonadaceae*, *Pseudomonas*
Physical characteristics
Gram-negative, rod-shaped bacilli
Motile via motile polar flagella
Non-spore-forming organisms
Produce a fluorescent pigment (pyocyanin), from which the name *P. fluorescens* is derived
Produce exopolysaccharides and readily form biofilms
Growth characteristics
Obligate aerobes but capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration
Optimal temperatures for growth
25–30°C for environmental isolates
34–37°C for mammalian isolates
Oxidase positive
Catalase positive
Grow well on Trypticase soy agar (TSA) and Luria agar (LA)
Hemolytic activity on red blood cells
No for environmental isolates
Yes for certain mammalian isolates (e.g., strain MFN1032)
Form small, white, convex colonies

TABLE 4 Summary of information on fully sequenced bacterial strains from the *P. fluorescens* species complex[a]

Strain	Isolation source	Genome size (Mb)	% G+C	Yr isolated/yr sequenced	GenBank accession no.	Reference(s)
P. fluorescens strains						
Pf0-1	Loam soil, Sherborn, MA	6.44	60.5	1988/2009	NC_007492.2	23, 221
SBW25	Sugar beet phyllosphere, Oxfordshire, UK	6.72	60.5	1989/2009	NC_012660	23
A506	Pear phyllosphere, California	6.02	59.9	1994/2012	NC_017911	49, 222
Q2-87	Wheat rhizosphere, Washington State (same field as that for Q8r1-96)	6.37	60.6	1987/2012	NZ_CM001558.1	49, 223
Q8r1-96	Wheat rhizosphere, Washington State (same field as that for Q2-87)	6.6	61	1996/2012	NZ_CM001512.1	49, 224
SS101	Wheat rhizosphere, near city of Bergen op Zoom, The Netherlands	6.18	60	2003/2012	NZ_CM001513	49, 64
WH6	Rhizosphere of *Poa* sp. and *Triticum aestivum* at Hyslop Research Farm, Benton County, OR	NA	NA	2008/2010 (draft)	NA	225, 226
F113	Sugar beet rhizosphere	6.85	60.8	1992/2012	NC_016830	50, 158
R124	Tepui orthoquartzite sandstone cave in Guiana Shield, South America	6.3	NA	2007/2013	NZ_CM001561	227
NCIM 11764	Culture supplied with potassium cyanide as the sole nitrogen source	6.97	59	1983/2012	NA	232, 233
P. protegens strains						
Pf-5	Soil, Texas	7.07	63.3	1978/2005	NC_004129.6	228
CHA0	Tobacco roots, Morens, Switzerland	6.87	63.4	1983/2013	NC_021237.1	229, 230
P. brassicacearum subsp. *brassicacearum* NFM421	Plant rhizosphere	6.84	60.8	NA/2011	NC_015379.1	231
P. chlororaphis subsp. *aureofaciens* strains						
30-84	Wheat rhizosphere, Kansas	6.67	62.9	NA/2012	NZ_CM001559	49
O6	Soil, Utah	6.98	62.9	1996/2012	NZ_CM001490	49, 234
P. synxatha BG33R	Peach rhizosphere, South Carolina	6.3	59.6	1993/2012	NZ_CM001514	49, 235

[a] NA, not available.

is ironic that taxonomy within the *Pseudomonas* genus is undergoing reorganization in the genomic era (77). Molecular methods, including analysis of 16S rRNA gene sequences, other highly conserved "housekeeping" genes, and, more recently, full-length genomes, have accelerated the pace of taxonomic reorganization, especially within the *P. fluorescens* species complex (76, 78–80). Multilocus sequence typing (MLST) and multilocus sequence analysis (MLSA) utilize the concept of genetic evolution at multiple conserved genes to measure evolutionary distances between species or strains (81–83). These conserved ("housekeeping") genes are required for the basic functions of the cell and must be found in all bacteria in the comparison (84–86). Examples of housekeeping genes used in classifying *Pseudomonas* species include *rpoD* (σ-subunit of RNA polymerase), *rpoB* (β-subunit of RNA polymerase), and *gyrB* (β-subunit of gyrase, responsible for negative supercoiling of DNA during replication) (80, 87, 88). The combination of these three housekeeping genes and the 16S rRNA gene was used to identify members of the *Pseudomonas* genus, create a phylogenetic tree, and divide them into different groups (74, 76) (Fig. 2). One of the key findings of these analyses is that the widest range of genomic diversity in the *Pseudomonas* genus is found in the *P. fluorescens* species complex (74) (Fig. 2).

The *P. fluorescens* species complex includes at least 52 separately named species, including *P. poae*, *P. synxatha*, *P. tolaasii*, *P. brassicacearum*, *P. chlororaphis*, and *P. fluorescens* (23, 60, 61). The *P. fluorescens* species complex can also be divided into three smaller taxonomic clades, based on 16S rRNA gene and MLST analyses (23, 49, 74, 89, 90) (Fig. 4). Silby et al. and Loper et al. have published comparative genomic analyses of three and seven bacterial strains, respectively, within the *P. fluorescens* species complex (23, 49). One approach to studying the level of genetic diversity between *P. fluorescens* strains is via the size of the pan-genome, which is the total number of genes found across all strains. While the pan-genome of *P. aeruginosa* is 7,824 genes, the pan-genome of *P. fluorescens* bacteria is much larger, at 13,782 genes. Silby et al. noted that the shared average nucleotide and amino acid identities of the three *P. fluorescens* genomes in their study (SBW25, Pf-5, and Pf0-1) were below those of the threshold for a "species" reported by Goris et al. (90). The study by Loper et al. included the original names of the *P. fluorescens* strains in the analysis but pointed out that their phylogenetic and comparative genomic analyses support the possibility that many of these species names will change in the future.

These two studies of full-length genomes confirmed the high genetic diversity within this group of bacteria. The analysis by Loper et al. (49) included a multiway BLASTp analysis to compare the seven newly sequenced *P. fluorescens* species complex genomes to previously annotated and sequenced genomes. An E value cutoff of 10^{-15} was selected to identify putative orthologs between the different strains (for DNA-DNA searches, E values of $<10^{-10}$ are

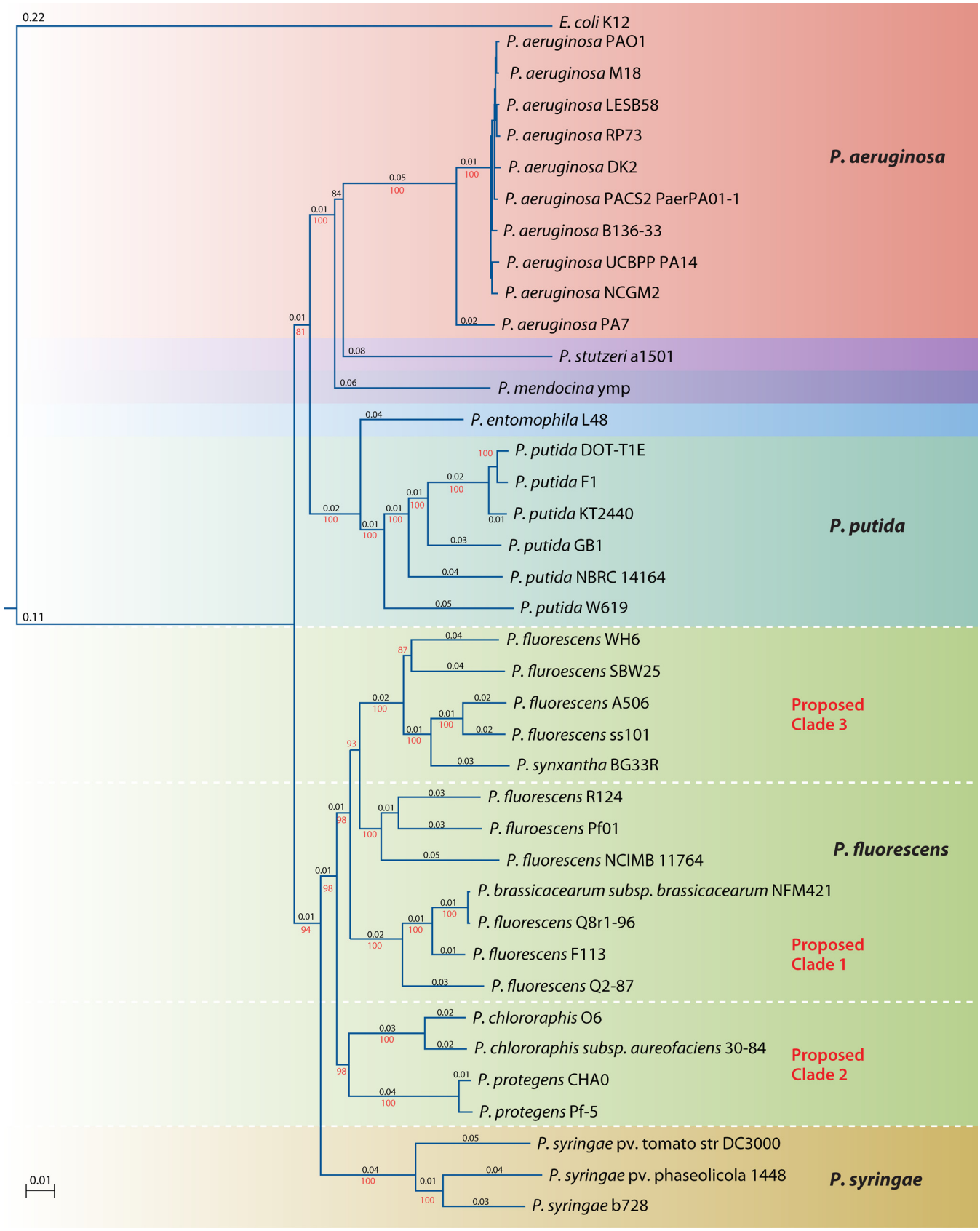

FIG 4 Phylogenetic tree of 38 *Pseudomonas* type strains, based on a concatenated nine-gene MLST analysis. The strains selected have full-genome sequences available through public databases. The MLST analysis was performed using nine housekeeping genes (encoding DnaE, PpsA, RecA, RpoB, GyrB, GuaA, MutL, PyrC, and AcsA), with *E. coli* strain K-12 used as the outgroup. A maximum likelihood tree was calculated in the online version of MAFFT (209, 210) and visualized with the software program Archaeopteryx (211). The confidence intervals after 1,000 bootstrap resamplings are indicated in red, and the branch distances are indicated in black. The bar indicates sequence divergence. *P. fluorescens* clade destinations are based on those proposed previously (49).

needed to provide evidence of homology and imply that the predicted homology would happen by chance only once in 10^{15} searches [91]). In the study by Silby et al. (23), a comparison of 14 *Pseudomonas* genomes (across multiple species) was performed all-against-all, using a reciprocal FASTA approach (30% identity over 80% of the length as the minimum similarity). While there is a "core genome" of 2,789 genes within the *P. fluorescens* species complex, only 20 are unique to the species complex itself within the *Pseudomonas* genus, and these encode proteins involved in regulation, biofilm formation, or unknown functions (49). Within each clade of the *P. fluorescens* species complex, the level of genetic similarity between strains is higher, with 4,188, 3,729, and 3,893 shared conserved domains between members of clades 1, 2, and 3, respectively (49).

The clade designation also offers some potential insights into functional differences between clusters of *P. fluorescens*, including the presence/absence and type of type III secretion system (T3SS), a molecular "needle" complex utilized by bacteria to inject bacterial proteins into host cells (49, 92). Genes for a T3SS are found only in clades 2 and 3, not clade 1. The biosynthesis gene cluster to produce hydrogen cyanide, a volatile molecule used to kill off competing bacteria, is found only in clades 1 and 2 (29, 33, 49). Genes found in every clade, such as those for the siderophore pyoverdine (93, 94), reflect functional categories that are generally preserved across the *Pseudomonas* genus as a whole (49). Comparative genomic analysis of *P. fluorescens* is in its early stages, but since there are already marked differences in the presence/absence of numerous genes between strains, this approach holds significant promise as a step in organizing the *P. fluorescens* species complex according to putative functional differences.

Identifying *P. fluorescens* in Samples by High-Throughput Sequencing

The coupling of high-throughput sequencing with the generation of 16S rRNA gene amplicon libraries from metagenomic samples has fueled the explosion in information about the microbiome and environmental microbial communities. Databases for subsequent bioinformatic analysis have continued to expand at a staggering pace. Historically, taxonomic assignment of a short read sequence from this type of analysis was limited to the family or genus level. However, as additional fully sequenced genomes become available to build validated phylogenetic trees of short read sequences, some genera can be resolved at the species level. This is turning out to be the case for some of the species in the *Pseudomonas* genus; the groups identified by MLST and MLSA can also be identified using the V3-V5 region of the 16S rRNA gene. As illustrated in Fig. 5, a phylogenetic tree can be generated using the16S rRNA gene sequences corresponding to the V3-V5 regions of the gene and a progressive tree alignment strategy (95–98). The bootstrap values for separating *P. aeruginosa* from the other *Pseudomonas* species are very high. While the bootstrap values are much lower for distinguishing the non-*aeruginosa Pseudomonas* species based on the V3-V5 region alone, the short-read, high-throughput sequencing technologies that target the V3-V5 variable region of the 16S rRNA gene can offer a first-pass analysis that discriminates between members of the *P. putida* and *P. fluorescens*/*P. syringae* clusters.

We have used this type of analysis, combined with other data, to demonstrate that both *P. aeruginosa* and *P. fluorescens* are prominent members of the respiratory microbiota of lung transplant recipients but that increases in their relative proportions are associated with widely divergent clinical associations (3). Multiple independent studies identified the presence of *P. aeruginosa* in respiratory cultures as a positive risk factor for the subsequent development of bronchiolitis obliterans syndrome (BOS) (99–101). However, in the largest published study of lung transplant subjects to date, utilizing high-throughput sequencing for microbial identification, a negative association was reported between the presence of *Pseudomonas* species and the diagnosis of BOS (102). In our study (3), we similarly observed high levels of *Pseudomonas* in lung transplant recipients (as determined by high-throughput sequencing of V3-V5 16S rRNA gene amplicon libraries). However, after applying the analysis described above and adding our *Pseudomonas* operational taxonomic units to the phylogenetic tree to delineate *P. aeruginosa* versus *P. fluorescens*, subjects with abundant *P. aeruginosa* had other clinical symptoms consistent with an acute infection, including positive *P. aeruginosa* bacterial cultures. In contrast, the numerous subjects with abundant *P. fluorescens* bacteria exhibited little evidence of acute infection, and no *Pseudomonas* species was detected via standard clinical laboratory bacterial culture. We alluded earlier in this review to the gap in knowledge about the factors that control culturability of *P. fluorescens* from clinical samples, which was underscored in our study. The surprising stark difference in culture positivity between these pseudomonads may explain the difference between prior culture-based studies (99–101) and the culture-independent study (102). Note that healthy controls in our study had very little signal for either *P. aeruginosa* or *P. fluorescens* in their bronchoalveolar lavage fluid (3). We provide this as an example of the potential power of high-throughput sequencing to provide new insights into the association of *P. fluorescens* with humans during health and disease.

FACTORS AFFECTING HOST COLONIZATION AND PERSISTENCE

Antibiotics and Secondary Metabolites

P. fluorescens produces a long list of secondary metabolites that allow it to successfully vie with competing microorganisms. Examples include phenazine (26–28), hydrogen cyanide (HCN) (29), 2,4-diacetylphloroglucinol (DAPG) (30, 31), rhizoxin (32–34), and pyoluteorin (35, 36). Phenazines can be produced by Gram-negative bacteria found in soil and marine environments, with *Pseudomonas* spp. being one of the major producers (28). Phenazines are pigmented compounds that have antitumor, antimalarial, antiparasitic, and antimicrobial activities (26). *P. fluorescens* produces the yellow phenazine phenazine-1-carboxylic acid (PCA) (28). Hydrogen cyanide is a volatile, colorless compound that inhibits cytochrome *c* oxidases and other metalloproteins in competing bacteria (33). The production of HCN by rhizosphere-inhabiting *P. fluorescens* suppresses plant disease (29). While it has not been studied for *P. fluorescens*, other *Pseudomonas* spp. are capable of producing HCN during human disease, such as cystic fibrosis (103). The anaerobic regulator protein ANR regulates the *hcnABC* gene cluster, which encodes hydrogen cyanide synthase, and, due to the oxygen sensitivity of the synthase, ensures that the genes are expressed only under low-oxygen conditions (104). DAPG production plays a significant role in the plant disease control activity of many *P. fluorescens* strains (30). Despite its importance, the DAPG biosynthetic cluster (*phl*) has been lost from all

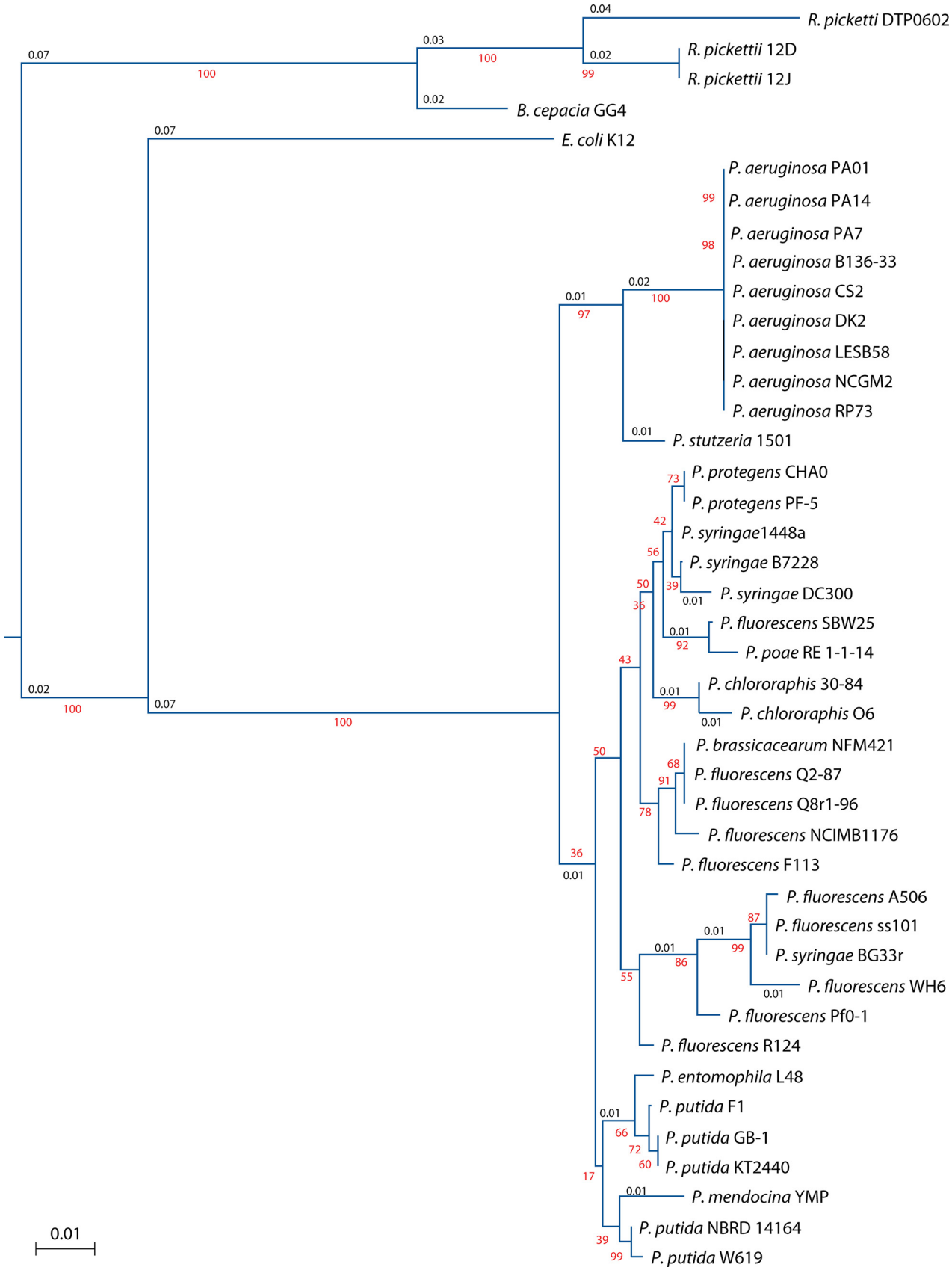

FIG 5 Phylogenetic tree of 38 *Pseudomonas* type strains, based on the V3-V5 region sequence of the 16S rRNA gene (V3 primer, positions 442 to 492; and V5 primer, positions 822 to 879 [numbered according to the *E. coli* 16S rRNA gene map]). The strains selected have full-genome sequences available through public databases. The V3-V5 sequence primers (212) were aligned to each genome by using DNAstar SeqBuilder software. A maximum likelihood tree was calculated in the online version of MAFFT (209, 210) and visualized with the software program Archaeopteryx (211). The confidence intervals after 1,000 bootstrap resamplings are indicated in red, and the branch distances are indicated in black. The bar indicates sequence divergence.

but a subset of *P. fluorescens* strains through evolution (31). Members of clades 1 and 2 of the *P. fluorescens* species complex (such as *P. protegens* Pf-5, *P. fluorescens* Q8r1-96, and *P. fluorescens* Q2-87) have retained the DAPG biosynthesis cluster, while all members of clade 3 do not possess this cluster (49). Intragenomic recombination and rearrangement occur frequently at this locus, such that DAPG-producing strains often have multiple versions of the *phl* gene cluster. In the phylogenetic lineage that retains DAPG synthesis, the gene cluster has maintained its structure, even though it has been relocated multiple times in the various *P. fluorescens* genomes (31). Rhizoxins are 16-membered macrocyclic lactones that interfere with microtubulin dynamics during mitosis by binding to β-tubulin (32) and that show inhibitory activity against fungi, bacteria, and tumors (33, 34). The rhizoxin-producing gene cluster in *P. fluorescens* is shared with another gammaproteobacterial genus, *Burkholderia* (105). Pyoluteorin was first isolated from a *P. aeruginosa* strain (106) but is now known to be produced by multiple *Pseudomonas* spp., including *P. fluorescens* (35). It has been studied in *P. fluorescens* strains Pf-5 and CHA0 for its antibacterial activity and ability to improve plant health (35, 36). While the activities of these secondary metabolites on human hosts remain to be determined, they benefit the survival of *P. fluorescens* in polymicrobial environments, opening the possibility of a role for these metabolites in survival of *P. fluorescens* in the human microbiome.

Other secondary metabolites produced by *P. fluorescens*, notably pyrrolnitrin and the pseudomonic acids, have been formulated for medical and agricultural uses. Pyrrolnitrin, a chlorinated molecule with antifungal activity, was developed into both a topical antimycotic for clinical use (39) and a fungicide for agricultural use (107). Pseudomonic acids are perhaps the most clinically important antibacterials produced by *P. fluorescens*. There are multiple pseudomonic acids (108), and each exhibits some level of antibacterial activity. Pseudomonic acid A has the highest activity and is the major pseudomonic acid (90%) in mupirocin, a topical antibiotic (40, 41, 109, 110). Topical mupirocin (2% concentration) is effective for treatment of superficial skin infections, such as impetigo, caused by the Gram-positive bacteria *Staphylococcus* spp. and *Streptococcus* spp. and the Gram-negative bacteria *Haemophilus influenzae* and *Neisseria gonorrhoeae* (41, 110–112). Pseudomonic acid A interacts with the amino acid binding site of isoleucyl-tRNA synthase and the respective ATP binding site, inhibiting the ability of bacteria to produce isoleucyl-tRNA synthetase (113–115). This inhibits protein synthesis primarily and RNA and bacterial cell wall synthesis to a lesser extent, possibly due to auxotrophy of amino acids that are important for these processes. The result is death of the bacterial cell. *P. fluorescens* is protected from pseudomonic acid because the *P. fluorescens* isoleucyl-tRNA target synthetase is structurally different and binds to pseudomonic acid with a much lower affinity (116). Overall, the production of pyrrolnitrin and the pseudomonic acids provides *P. fluorescens* with significant growth advantages in polymicrobial environments.

Siderophores and Pigments

The secretion of a fluorescent pigment, pyoverdine (formally called fluorescein), is what imparts *P. fluorescens* with its fluorescence properties under UV light. Pyoverdine is a siderophore (117), a high-affinity iron-chelating compound that is essential for acquisition of iron from the environment, bacterial growth, and survival (43). Pyoverdine is the main siderophore of *P. fluorescens* (93), but some strains of *P. fluorescens* contain additional secondary siderophores for iron acquisition. *P. protegens* CHA0, a *P. fluorescens* species complex strain, produces the secondary siderophore enantio-pyochelin (44), and *P. fluorescens* strain ATCC 17400 produces the secondary siderophores quinolobactin, pseudomonine, acinetobactin, and anguibactin (118). The last three secondary siderophores from this strain are synthesized through a single pathway, with different primary substrates determining which final siderophore molecule is synthesized (45). Strains BG33R (*P. synxantha*) and A506 (*P. fluorescens*) also have the gene clusters necessary for the biosynthesis and uptake of a pseudomine-like molecule similar to that found in ATCC 17400, but the functionality of this molecule has not yet been demonstrated (49, 119). The full complement of genes necessary for the biosynthesis and efflux of a hemophore, which allows for the chelation and transport of heme through a specific outer membrane (49), are also present in multiple *P. fluorescens* strains, but it is not known how and when hemophores are utilized by *P. fluorescens*. Much work remains on identifying the spectrum of siderophores produced by *P. fluorescens* strains, as well as determining their role in the physiology of these organisms under different conditions, including polymicrobial competition.

Like many other members within its genus, *P. fluorescens* produces a range of pigments, with and without siderophore ability. Due to the ability of *P. fluorescens* to grow at temperatures as low as 4°C, contamination of food products can be a problem (120). In 2010, European consumers noticed that some mozzarella products were blue instead of white, and extremely high levels of *P. fluorescens*, up to 10^6 CFU/g, were identified on the "blue" cheese samples (121). Beyond being blue, little is known about this particular pigment produced by *P. fluorescens*. Pyocyanin, another blue pigment, is produced by *P. aeruginosa*, but this secondary metabolite has not yet been identified in *P. fluorescens* (122). This incident indicated either the emergence of a new strain of *P. fluorescens* that had acquired the biosynthesis machinery for a new blue pigment or horizontal acquisition of the biosynthesis machinery from another, closely related *Pseudomonas* strain.

Two-Component Gene Systems

P. fluorescens also contains a two-component GacS-GacA system that plays a role in environmental sensing. This system controls the expression of multiple secondary metabolites and enzymes in *P. fluorescens*, including DAPG, pyoluteorin, HCN, phospholipase C, and exoprotease (123–126). In *P. aeruginosa*, GacA controls gene expression through acylated homoserine lactone (AHL) signaling (127, 128). However, GacA can also function independently of AHL signaling (127), and this AHL-independent GacA cascade has been reported for *P. fluorescens* strain CHA0 (129). The diffusible non-AHL bacterial signal, whose chemical nature is still under investigation, turns on and regulates a two-component GacS-GacA system that activates the transcription of a novel small, noncoding RNA, RsmY (129). RsmY then combines with a riboregulator (RsmA), which is a small, untranslated RNA that can regulate cellular processes (130–133), to positively regulate the expression of downstream genes at a posttranscriptional level (65).

Quorum Sensing and Biofilms

Bacteria are able to regulate their population density through the release and sensing of signal molecules, i.e., quorum sensing (134, 135). Quorum sensing involves regulation of genes that control motility (swimming and swarming), antibiotic synthesis, and biofilm formation. Genes involved in biofilm formation and quorum sensing are found in the core genome of the *P. fluorescens* species complex (49). Quorum sensing and biofilm formation are integral to the many environmental niches occupied by *P. fluorescens* and allow it to colonize surfaces such as hospital equipment and food-grade stainless steel surfaces (52, 136), as well as the surfaces of plants, showerheads, and even indoor wall surfaces (23, 24, 137). *P. fluorescens* readily forms biofilms with highly complex, three-dimensional (3-D) structures (Fig. 6) (20, 52–56), and strains that form plant-associated biofilms are often important biocontrol agents that protect plants against pathogenic fungi (54, 138). Less is known about *P. fluorescens* biofilm formation on mammalian surfaces, though the adaption to a 37°C permissive growth range is linked to biofilm formation on human cells (53). Thus, whether on plants or human cells, biofilm formation is likely important for successful long-term colonization by *P. fluorescens*.

Two types of quorum sensing systems have been described for *P. fluorescens*: the AHL/*lux* and *hdtS* systems. In Gram-negative bacteria, AHL molecules are produced by LuxI-like proteins and interact with LuxR-like proteins to form dual AHL-LuxR complexes. This AHL-LuxR complex then binds *lux* boxes of quorum sensing-regulated genes in order to either turn on/up or off/down their expression (139). A *luxI-luxR*-like system in *P. fluorescens* was first discovered in the strain NCIMB 10586 and was termed the *mpuI-mpuR* system due to its regulation of the antimicrobial mupirocin biosynthesis pathway (140). Another quorum sensing system, the *hdtS* system, was later discovered in *P. fluorescens* strain F113 (141). The *hdtS* gene encodes a novel AHL synthase that produces separate signaling molecules: an *N*-(3-hydroxy-7-*cis*-tetradecenoyl)homoserine lactone (3-OH-$C_{14:1}$-AHL), an *N*-decanoylhomoserine lactone (C_{10}-AHL), and a C_6-AHL. Though the signaling molecules and synthase have been elucidated, the genes regulated by the *hdtS* system are still unknown, and no detectable phenotype in F113 has yet been linked to the signaling molecules (141).

The second messenger cyclic di-GMP (c-di-GMP) is essential for regulation of steps involved in biofilm formation, including the production of LapA, an adhesive protein necessary for *P. fluorescens* attachment to surfaces (142). LapA is negatively regulated by the periplasmic protease LapG and positively regulated by the inner membrane protein LapD (143). LapG typically cleaves LapA from the bacterial surface, but when LapD is bound by c-di-GMP, LapD undergoes a conformation change that allows it to bind to LapG, inhibiting LapA cleavage. Diguanylate cyclases catalyze c-di-GMP synthase activity, and in *P. fluorescens* Pf0-1, there are a total of 43 potential diguanylate cyclases encoded in the genome, each potentially connected to a different aspect of biofilm formation (144).

Type III Secretion Systems

Type III secretion systems (T3SSs) are molecular needle-like complexes that act like syringes to deliver bacterial proteins, called effectors, from the bacterial cytoplasm directly into host cells (92) (Fig. 7). T3SSs are highly conserved genomic clusters typically found in bacteria that have close interactions with eukaryotic hosts (often transferred horizontally between phylogenetically unrelated bacteria), and the type of T3SS usually mirrors the type of interaction a bacterium has with the eukaryotes in its environment. The first T3SS was described for *Yersinia*, which delivers Yop (*Yersinia* outer protein) effector proteins into human host cells (145, 146). A total of five different T3SS groups have since been described: the Ysc group (which includes the *Yersinia* Ysc, *P. aeruginosa* Psc, *Bordetella* Bsc, *Rhizobium* Rsc, and *Chlamydia* sp. T3SSs), the Hrp1 group (found in non-*aeruginosa Pseudomonas* spp. and *Erwinia* spp.), the Hrp2 group (found in *Xanthomonas* spp. and *Ralstonia* spp.), the Inv/Mxi/Spa group (which includes the *Salmonella* SPI-I, *Shigella* sp., and *Yersinia enterocolitica* Ysa T3SSs and T3SS2 of enterohemorrhagic *E. coli* [EHEC]), and the Esa/Ssa group (including the *Salmonella* SPI-2 and enteropathogenic *E. coli* [EPEC] T3SSs and EHEC T3SS1) (147).

The Hrp1 family is the most common T3SS found among *P. fluorescens* strains (46–50). The Hrp (hypersensitivity response and pathogenicity) system triggers the hypersensitivity defense response in resistant plants, while leading to disease in susceptible plants, and was first described for *P. syringae* (148). Like the T3SS found in *Yersinia*, the Hrp1 system is involved in delivering bacterial proteins directly into host cells (149–152) (Fig. 7). While the fully sequenced *P. fluorescens* strains SBW25, BG33R, A506, SS101, Q8r1-96, and Q2-87 have at least one copy of the Hrp1 family T3SS, Pf0-1 and Pf-5 do not carry the gene cluster at all (49, 153). The activity and functionality of the Hrp1 system have been worked out for only a couple of the strains in which it has been found. The Hrp1 T3SS of *P. fluorescens* Pf29Arp, a strain known for its ability to reduce the severity of wheat take-all, shows activity during the colonization of wheat rhizospheres (46). The homologous Hrp1 T3SS in strain SBW25 is induced during sugar beet rhizosphere colonization (154) and can induce a hypersensitive response in tobacco (47, 155). Interestingly, in addition to Hrp1 system effectors, SBW25 also contains the T3SS effector ExoY (156), which in *P. aeruginosa* targets the actin cytoskeleton of eukaryotic cells (157). Since most of the work on the functionality of the Hrp1 T3SS in *P. fluorescens* has been done *in vitro*, many of the target host cells are still unknown, but the presence of the ExoY effector protein in some strains suggests that there might be an additional, nonplant use of this T3SS in SBW25 and genetically related strains.

Additional evidence that *P. fluorescens* strains may target their T3SSs against eukaryotic cells was provided in 2013, when a SPI-I-like T3SS gene cluster was discovered in strain F113 (51) (Fig. 7). The F113 strain was originally isolated from sugar beet rhizospheres in Ireland (158) and can inhibit the growth of plant-pathogenic bacteria, oomycetes, fungi, and a wide range of nematodes (159–161). Predation against protozoa in both terrestrial and aquatic environments is an important factor influencing bacterial community makeup and behavior (37, 38, 162). In F113, the SPI-I T3SS *hilA* promoter shows increased expression during close contact with the amoeba *Acanthamoeba castellanii*, suggesting that this T3SS is directly involved in protecting the bacterium from amoeba predation. Interestingly, both the Hrp1 and SPI-I systems in F113 appear to be involved in protection against predation by the worm *Caenorhabditis elegans* (51). A similar result was found with the Hrp1 system of *P. fluorescens* CHA0 (163). Additional SPI-I T3SSs have also been found in *P. fluorescens* strains HK44 (164) and Q2-87 (49), providing further evidence of T3SS action

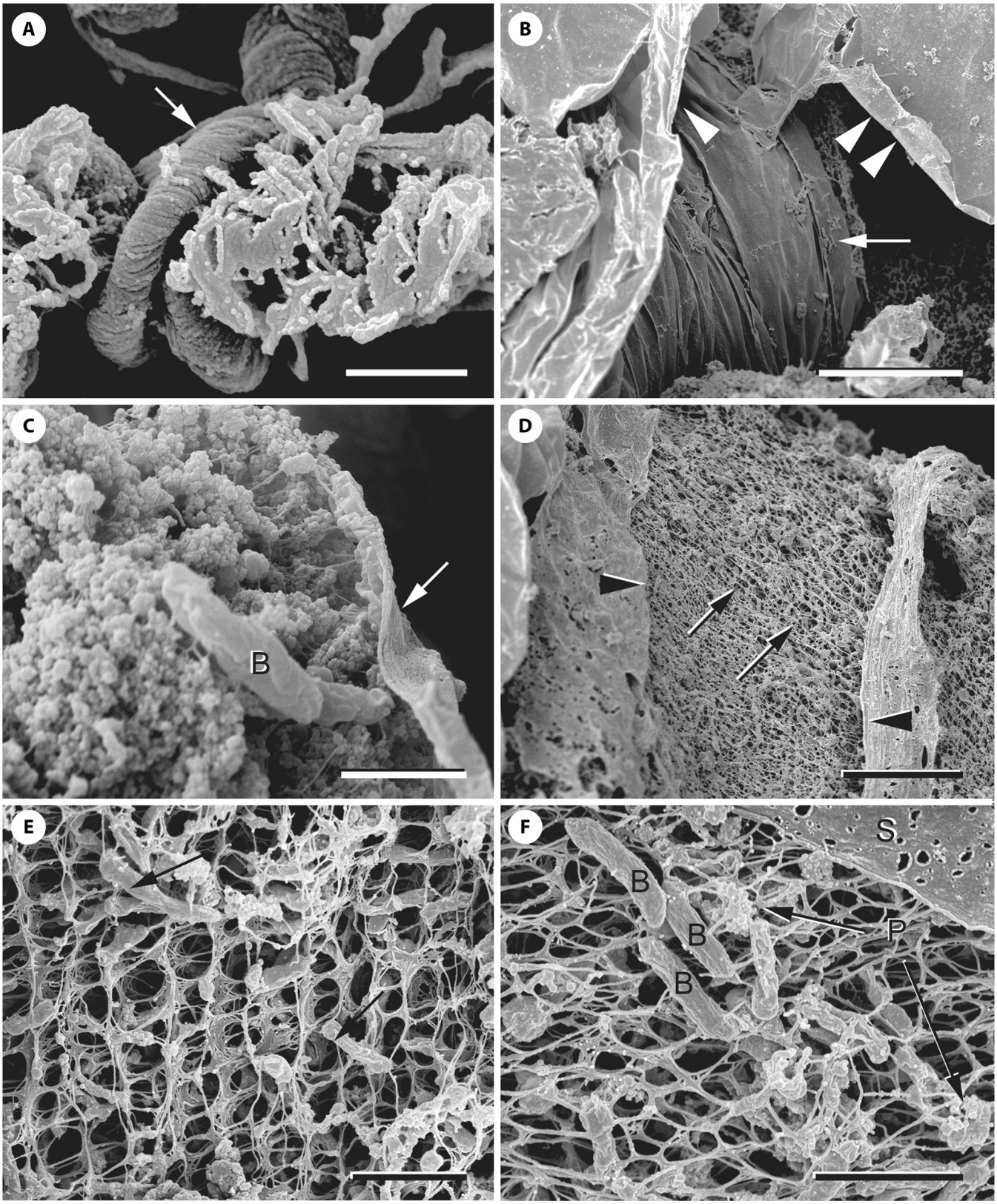

FIG 6 Scanning electron micrographs of *P. fluorescens* biofilms. For these photomicrographs, Baum et al. prepared and cryopreserved 14-day biofilms from *P. fluorescens* EvS4-B1 monocultures (56). (A) Fibrillary structures made up of twisted fibers (arrow). Bar = 1 μm. (B) Flat sheets of material (arrowheads), with some of the sheets wrapped around other structures (arrow). Bar = 20 μm. (C) The inside core of the "wrapped" structures, consisting of bacteria (B) embedded in an extracellular matrix of particulate matter, and a thin sheet of material (arrow). Bar = 1 μm. (D) The outer sheet (arrowheads), which envelops an inner core consisting of fibers forming irregular network-like structures (arrows). Bar = 10 μm. (E) Network consisting of fibers arranged in a periodic pattern, with bacteria (arrows) dispersed throughout the network. Bar = 2 μm. (F) A sheet of material (S), consisting of extracellular material and dead cells, covering and attaching to the fiber network and including associated bacteria (B) and particulate matter (P). Bar = 2 μm. (Reprinted from *BMC Microbiology* [56] under a Creative Commons license [http://creativecommons.org/licenses/by/2.0/].)

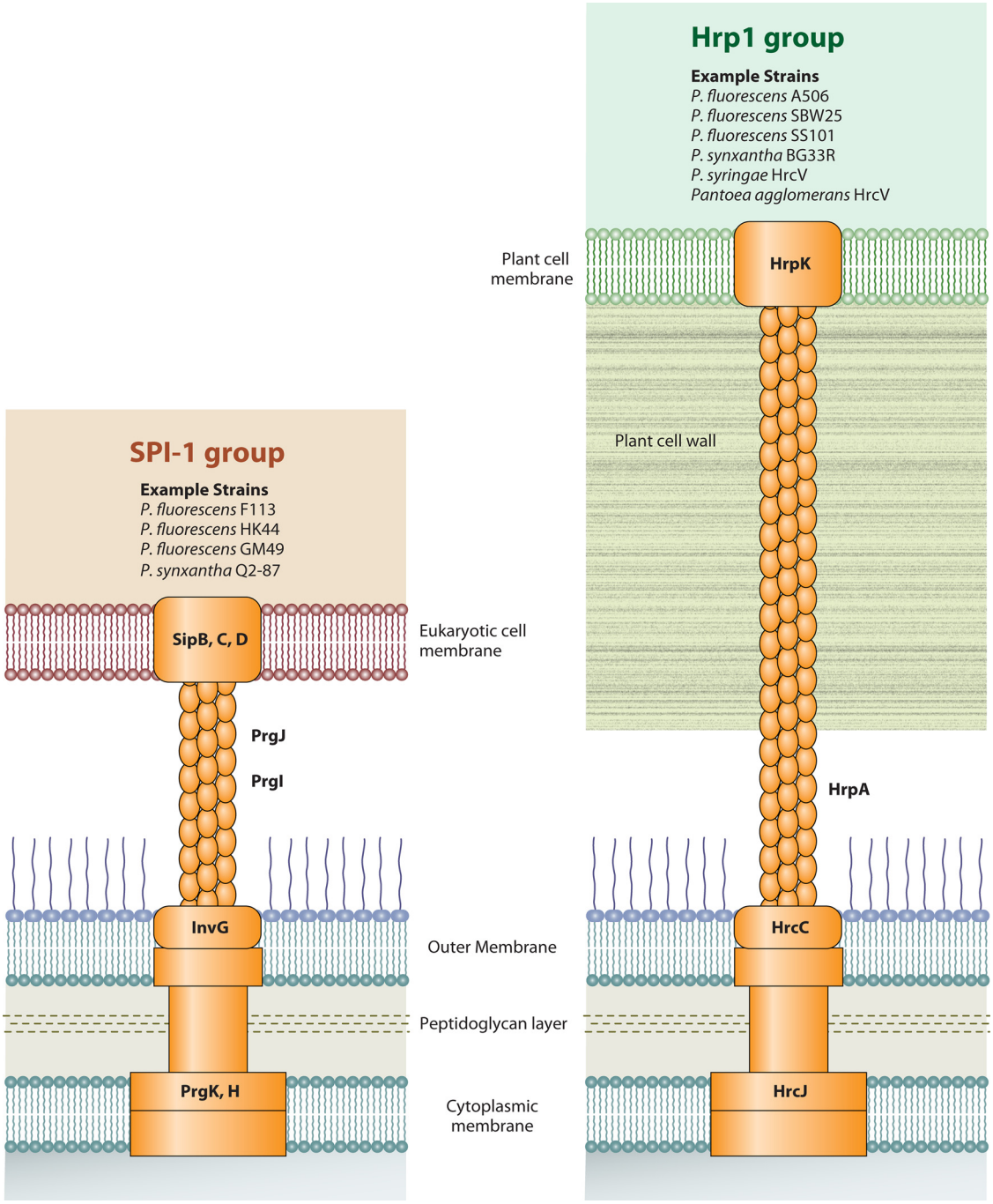

FIG 7 Type III secretion systems in *P. fluorescens*. The components and structures of the SPI-I and Hrp1 systems are shown, with lists of the corresponding strains in which these systems have been identified.

outside the plant ecosphere. Thus, the identification of multiple T3SSs across the *P. fluorescens* species complex that target plant and nonplant eukaryotic cells supports the model of a wider interaction of *P. fluorescens* with eukaryotic hosts.

INTERACTION OF *P. FLUORESCENS* WITH HUMAN CELLS

Environmental isolates of *P. fluorescens* have an optimal temperature growth range of 25 to 30°C and are not virulent to human cells, but certain strains of *P. fluorescens* isolated from clinical samples have a higher permissive growth range, up to 37°C, and show increased virulence against human cells (53, 57–59). Two *P. fluorescens* strains, MFY162 and MFN1032, can adhere to human glial cells in culture, and MFN1032 can induce apoptosis. Originally isolated from an individual with a lung infection (57), MFN1032 not only exhibits cytotoxicity on human intestinal epithelial cells *in vitro* but also triggers a proinflammatory response (165). Human airway epithelial cells exposed to a different strain of *P. fluorescens* have been shown to trigger both antiapoptotic responses,

via the epidermal growth factor receptor (EGFR), and interleukin-8 (IL-8) production, via Toll-like receptor 4 (TLR4)-independent NF-κB signaling pathways (166). Exposure to a strain of *P. fluorescens* isolated from a moldy building decreased viability of mouse macrophages (RAW cells) while inducing production of nitric oxide, tumor necrosis factor (TNF), and IL-6 (167).

On red blood cells, *P. fluorescens* MFN1032 displays both cell-associated and secretion-dependent hemolytic activity. The secretion-dependent pathway is positively regulated by the GacS-GacA two-component system (58), the same two-component system that regulates phase variation in this strain (168). This hemolytic activity involves the production of phospholipase C and biosurfactants, similar to that seen for pathogenic *P. aeruginosa* (169). Similarities between *P. aeruginosa* and *P. fluorescens* also exist within the functionality of the cell-associated hemolytic activity of MFN1032. The cell-associated hemolytic activity is independent of the secretion-association hemolytic activity, is active at 37°C, occurs without the secretion of phospholipase C and biosurfactants, and does not depend on the GacS-GacA two-component system (170). In *P. aeruginosa*, cell-associated hemolytic activity occurs alongside type III secretion of the PcrV, PopB, and PopD effectors (171). MFN1032 also harbors the genes necessary to produce a T3SS (170), the *hrcRST* gene cluster, which shares a high level of homology to the *hrcRST* genes of the *hrpU* operon in *P. syringae* DC3000. When this operon is mutated, MFN1032 is no longer able to produce cell-associated hemolytic activity (170). In *P. aeruginosa*, similar mutations in the T3SS also abolish its cell-associated hemolytic activity. Thus, adaptation of *P. fluorescens* MFN1032 results in an increased temperature permissivity along with hemolytic activity against human cells that is similar to that found in *P. aeruginosa*.

The production of cyclolipopeptides (CLPs) by *P. fluorescens* MFN1032 is another functional characteristic that is altered during a shift to higher temperatures. Cyclolipopeptides are the most widely studied biosurfactants produced by *P. fluorescens* and are involved in swarming motility, biofilm formation, and colonization of host surfaces (172). If MFN1032 is grown for multiple generations at 37°C, CLP functionality is lost, with $\sim 4 \times 10^{-3}$ CLP-deficient mutants found per generation (58). High mutation rates, inversions of DNA segments, DNA methylation, and epigenetic switches are all mechanisms that bacteria use to alter their genomes in the process of adaptation, which allows survival in changing environments and an increase in overall fitness with time (173). In the case of the *P. aeruginosa* T3SS, there is an epigenetic switch between a noninducible and an inducible state (168). Using a Boolean modeling system, a similar epigenetic switch has been shown to be the likely mechanism by which *P. fluorescens* regulates its CLP production (168). In much the same way that chronic *P. aeruginosa* strains lose the ability to produce biofilms after long-term growth in a cystic fibrosis lung (174), *P. fluorescens* also has a mechanism to turn off energy-expensive surfactant production after long-term growth at physiologically relevant temperatures.

CLINICAL SIGNIFICANCE

P. fluorescens as a Disease-Causing Agent

The bloodstream is by far the most common site reported for *P. fluorescens* infection (opportunistic) in humans. Most reported cases have been iatrogenic, with bacteremia attributable to either transfusion of contaminated blood products (7–12) or use of contaminated equipment associated with intravenous infusions (13–17). *P. fluorescens* bacteremia has occurred in outbreaks (8, 13–16), with the largest affecting at least 80 patients in 6 states after indirect exposure to contaminated heparinized saline flushes prepared at a common compounding pharmacy (16). Of these patients, 41% were bacteremic more than 84 days after exposure; all of these delayed-onset patients had indwelling ports for venous access, indicating that *P. fluorescens* can persist endovascularly when an indwelling catheter is in place. The abilities to grow at refrigerated temperatures and to form biofilms on fomite surfaces make *P. fluorescens* contamination a particular problem for blood infusion-related infections and outbreaks.

Confounding the diagnosis of *P. fluorescens* bacteremia is the well-described phenomenon of "pseudobacteremia" due to environmental contamination of blood culture collection bottles and equipment by the organism (175–182). Indeed, in a systematic review of the medical literature, more positive *P. fluorescens* blood culture results were attributable to pseudobacteremia (175–182) than to true bacteremia (8–17, 183–189). Sources have included blood culture bottles cleaned with contaminated disinfectant (179) and, most commonly, contaminated blood collection tubes used prior to culture bottle inoculation (176, 178, 180–182). Despite not reflecting "true" human pathology, pseudobacteremia is a legitimate clinical problem, resulting in diagnostic confusion for clinicians and inappropriate antibiotic exposure for patients (181). The diagnosis of pseudobacteremia should be considered when patient symptoms are discordant with disseminated bacterial infection and bacteria that are uncommon infectious agents (such as *P. fluorescens*) are isolated, especially in a geographic or temporal cluster.

Identification of *P. fluorescens* as an acute cause of infection (opportunistic or primary) in sites other than the blood has been rare and sporadic (Table 1). Two reports have identified *P. fluorescens* in skin wounds and abscesses following dog bites (190, 191), and in one instance, the patient subsequently developed disseminated *P. fluorescens* bacteremia (191). *P. fluorescens* has been implicated as a cause of acute bacterial cystitis (192–194), both with (192) and without (193) the presence of an indwelling urinary catheter. In a study comparing the oral microbiomes of 20 solid organ transplant recipients and 19 nonimmunosuppressed control subjects, *P. fluorescens* was abundant in the saliva of nearly 50% of transplant subjects while being nearly absent from nontransplant controls (1). In another study of 258 stomach wall biopsy specimens acquired from patients with various upper gastrointestinal disorders, 93% had evidence of the presence of *P. fluorescens* (identified via both culture-dependent and -independent methods) (2). Thus, *P. fluorescens* can clearly establish itself in diseased humans, but questions remain about the pathogenicity of such interactions and whether the involved strains are all restricted to a specific clade.

P. fluorescens in Respiratory Diseases

While *P. fluorescens* has repeatedly been cultured from respiratory specimens, its role in pneumonia or other respiratory infections is unclear. *P. fluorescens* has been cultured from the tracheal aspirates of patients receiving mechanical ventilation and subsequently identified as an organism in the humidifier water used in the ventilator circuit (195), but it is unclear if the tracheal aspirate culture results reflected acute infection or benign colonization. In

another case study, during recovery from a recent polymicrobial peritonitis, a patient developed clinical evidence of pneumonia, with sputum cultures that were positive for *P. fluorescens* (196). The patient improved after treatment with a third-generation cephalosporin, and subsequent sputum cultures did not grow *P. fluorescens*. In another report, *P. fluorescens* is mentioned in the etiology of community-acquired pneumonia in a single patient, but clinical details are lacking (197). Using amplification of bacterial 16S rRNA genes, another study detected *P. fluorescens* and other bacteria in the bronchoalveolar lavage fluid acquired from a single patient with clinically diagnosed ventilator-associated pneumonia (198). Most notably, in a survey of over 1,000 respiratory cultures acquired from subjects with cystic fibrosis, Klinger and Thomassen identified the organism in roughly 2% of specimens (199) and considered the organism a colonizer rather than an acute pathogen. We have reported, using bronchoalveolar lavage fluid acquired from lung transplant recipients, that *P. fluorescens* is frequently identified in this patient population, in the absence of evidence of acute infection (3).

In a survey of bacterial culture isolates at the University of Michigan Hospital, *P. fluorescens* was cultured from respiratory specimens with relative frequency (3) (Table 2). Over an 11-year period, *P. fluorescens* was cultured from over 240 distinct respiratory specimens, or roughly 2 specimens per month. Among patients with positive *P. fluorescens* respiratory cultures, the most common underlying pulmonary condition was cystic fibrosis (38.8% of all isolates), followed by other chronic airway diseases (chronic obstructive pulmonary disease [COPD], asthma, and non-cystic-fibrosis bronchiectasis [16.1%]). *P. fluorescens* was often coisolated with other organisms, most often (85.1%) species designated "oral flora" by the clinical microbiology laboratory, followed by *P. aeruginosa* (25.6%), *Staphylococcus aureus* (15.7%), and *Stenotrophomonas maltophilia* (11.6%). In no cases was *P. fluorescens* the unambiguous causative agent in a monomicrobial pneumonia. This survey highlights the fact that *P. fluorescens* is commonly isolated from human clinical samples in cases where it is not the cause of active acute infection. This contrasts with much of the literature, which states that *P. fluorescens* is found only in human hosts in extreme cases of outbreak or contamination. In addition, no reports were created in response to these *P. fluorescens* cultures, revealing that the number of reports in the literature also likely do not reflect the consistency with which *P. fluorescens* is cultured from clinical samples.

P. fluorescens and Inflammatory Bowel Disease

P. fluorescens has also been speculated to have a possible role in the pathogenesis of Crohn's disease and other inflammatory conditions. I2, a peptide encoded by *P. fluorescens*, was found to be detected more frequently in gut wall biopsy specimens of patients with Crohn's disease than in those of patients with other bowel diseases, and a similar difference was noted in detection of circulating anti-I2 antibodies (200). Interestingly, there was no evidence of *P. fluorescens* in the stool of subjects with Crohn's disease, by either culture or microbe-specific PCR. The same I2 sequence was also found in the proximal colon, cecum, and distal small intestine in C57BL/6J mice, suggesting that *P. fluorescens* can exist in the intestinal microbiota of multiple mammalian species (201). In TLR4- and MyD88-knockout mice that were treated with dextran sodium sulfate, the resulting colitis and impaired immune response led to systemically detectable *P. fluorescens*, such that it could be cultured from the mesenteric lymph nodes (202). Approximately 50% of Crohn's disease patients develop serum anti-I2 antibodies, and in some studies, this seroreactivity has correlated with the success of therapies aimed at the microbiome rather than the immune system (18–22). I2 is encoded within the *P. fluorescens pfiT* gene and has T-cell superantigen activity (203). The presence of anti-I2 serum antibodies in Crohn's disease patients was subsequently shown to be positively associated with the prognosis (19). Anti-I2 antibodies have also been associated with the diagnosis of celiac disease, including a decrease in titer after a gluten-free diet is initiated (204, 205), of ankylosing spondylitis (206), and of chronic granulomatous disease (207). Whether *P. fluorescens* directly contributes to these chronic inflammatory conditions or whether anti-I2 antibodies are only indirect biomarkers of disease is undetermined.

FUTURE PERSPECTIVES

Despite being identified in the last half of the 1800s and more recent associations with human disease, the role of the *P. fluorescens* species complex in human health and disease remains largely unexplored. Research in the last 2 decades on the genetic, molecular, environmental, and immunological aspects of the *P. fluorescens* species complex has begun to expand our understanding of these bacteria overall and to lay the groundwork for investigating their role in human health. Full-genome sequencing and comparison led to the discovery of potential pathogenic traits (such as T3SSs and T-cell superantigens) and further revealed the high level of genetic diversity within the *P. fluorescens* species complex. The discovery of human-adapted *P. fluorescens* strains with higher permissive temperature ranges revealed that these bacteria can readily exist outside plant and soil niches, and even potentially change their functional phenotypes in response to a new, mammal-based niche. Clinical surveys have also found that *P. fluorescens* is regularly cultured from clinical samples even in the absence of acute infection or outbreak. Studies are beginning to identify *P. fluorescens* via high-throughput sequencing in multiple sites of the human body, suggesting that the human-*P. fluorescens* connection will only grow as more studies are reported.

However, there is still much more that is unknown about the role of the *P. fluorescens* species complex in human disease. Taxonomic classifications within the *P. fluorescens* species complex are still in flux; a general consensus on what constitutes a *P. fluorescens* strain would codify classification and greatly assist in functional microbiology research, as well as the clinical microbiology lab and clinician. Almost nothing is known about the host response to *P. fluorescens*, and while correlations have been found between *P. fluorescens*-specific antibodies and Crohn's disease, the mechanisms underlying this connection have not been identified. Finally, there is a glaring disparity between reports in the medical literature that only find *P. fluorescens* infections during outbreaks/extreme situations and clinical surveys that readily identify *P. fluorescens* in human samples in the absence of acute disease. The former suggest that *P. fluorescens* is accidentally associated with human hosts through contamination or when the host is immunocompromised; the latter suggest that there are strains of *P. fluorescens* that can colonize and thrive in a human host. Additional work on the genomics, molecular microbiology, and host immune response to the *P. fluorescens* species complex will provide insight into the roles these bacteria play in human health and disease.

ACKNOWLEDGMENTS

The following funding sources have provided research support for the authors: The National Institute of Allergy and Infectious Diseases (B.S.S. [grant T32AI007528]), The National Heart, Lung and Blood Institute (G.B.H. [grants U01HL098961 and R01HL114447] and R.P.D. [grant T32HL00774921]), The Cystic Fibrosis Foundation (J.J.L.), and The Nesbitt Program for Cystic Fibrosis Research (J.J.L. and G.B.H.).

We thank the following individuals for their contributions to the manuscript: Karin J. Ekholm for translation of the Baader 1887 reference, Ellen Hunter for her assistance with the photographs of the Gram stains, and Patrick Lane for his assistance with the graphics.

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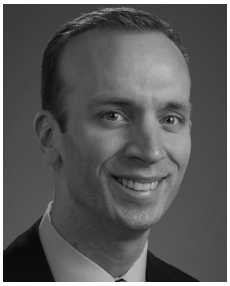

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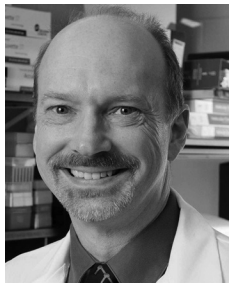

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