

The Formation of Biofilms

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1. Introduction

Biofilms are matrix-enclosed bacterial populations that are adherent to each other and to surfaces and/or interfaces and are mainly composed of polysaccharides, proteins, lipids, and extracellular DNA ^{[1][2][3]}. During biofilm formation, the cells transit from a motile to a sessile lifestyle by interacting with a surface and starting to produce an extracellular matrix that holds them together and attaches them to the surface ^[2]. Therefore, the cells forming biofilms are referred to as sessile cells, which differ from their non-encased free-swimming counterparts, the planktonic cells ^[4]. Recent studies indicate that biofilms represent the main mechanism of active bacterial life due to their dominance in all habitats throughout the world ^{[5][6]}. Compared to the planktonic lifestyle, the biofilm lifestyle confers several benefits to the integrating cells, such as protection against antimicrobial agents and predators, tolerance towards changing environmental conditions, and colonization aptitudes ^{[3][7][8]}.

Bacteria form biofilms in artificial and natural environments, including the soil, internal and external tissues of all living organisms, rocks, and water, among others ^[5]. Many different bacteria form biofilms, but the *Pseudomonas* genus is among the most studied for several reasons: (1) it harbors species with the ability to colonize a wide variety of environments due to the high metabolic and physiologic versatility found in this group of microorganisms, (2) it has ecological relevance due to its interactions with living organisms, and (3) it has potential biotechnological applications ^[9]. The *Pseudomonas aeruginosa* species, a ubiquitous bacterium that can also act as an opportunistic human pathogen, has long been used as a model bacterium within the *Pseudomonas* genus for the study of biofilm formation and pathogenesis due to its relevance in the clinical environment ^[10]. The extracellular matrix of *P. aeruginosa* has been studied in-depth and, to date, is known to contain three exopolysaccharides: alginate, polysaccharide synthesis loci (Psl), and pellicle loci (Pel) ^[11]. The role of these exopolysaccharides in the biofilm architecture of *P. aeruginosa* and the impact of their production in the clinical setting, such as protection against antibiotic treatments and host defenses, have been explored in several studies ^{[12][13][14][15][16][17][18][19][20]}. Although *P. aeruginosa* produces infections in humans, there are also some examples in which this bacterium can act as a pathogen for plants ^{[21][22]}. However, the biological significance of alginate, Psl, and Pel exopolysaccharides in a nonclinical context has not been studied.

Bacteria belonging to the *Pseudomonas* genus are common inhabitants of plant surfaces ^{[23][24]}. The role played by *Pseudomonas* in the agricultural industry is remarkable as several economically important activities are derived from their interaction with plants. Among these activities, there are harmful diseases that involve severe economic losses and beneficial activities such as plant growth stimulation, the promotion of plant health and nutrient availability in soils, and induction of plant immune defenses ^{[25][26]}. Pathogenic plant-associated *Pseudomonas* are predominantly present on the phyllosphere. The phyllosphere is an extreme and unstable habitat as it is exposed to highly variable nutrient and water availability, temperatures, and ultraviolet (UV) radiation. Therefore, the microbial populations associated with the phyllosphere must be adapted to these continuously fluctuating conditions ^{[23][27][28]}. The extracellular matrix of epiphytic bacteria contributes to the fitness ^{[29][30][31]}, protection ^{[8][32]}, and hydration of the cells ^[33], allowing cells to cope with these ever-changing conditions. Conversely, beneficial plant-associated *Pseudomonas* usually prevail in the rhizosphere. Compared to the phyllosphere, the environmental fluctuations that take place on the rhizosphere are weak and buffered ^[34]. Nevertheless, the rhizosphere is not considered a uniform and stable environment as the conditions can change abruptly in extremely short distance ranges ^{[35][36]}. Biofilm formation by beneficial plant-associated *Pseudomonas* plays advantageous roles for both the plant and bacteria ^{[27][37]}. On the one hand, they can increase plant yield by improving mineral uptake and phytohormone production, inducing the competitive suppression of pathogens and triggering plant-induced systemic resistance ^[38]. On the other hand, these biofilms allow the attachment of the cells to a nutrient source

and confer protection against plant defenses and environmental fluctuations [27][37]. Furthermore, the biofilms produced by rhizospheric bacteria enhance soil aggregation, which improves the water-holding capacity, fertility, and porosity of the soils, leading to an increase in agricultural productivity [39][40][41][42].

Some of the biofilm components, mainly exopolysaccharides, that are required for biofilm formation and pathogenesis in *P. aeruginosa* find their equivalents in pathogenic and beneficial plant-interacting *Pseudomonas*.

2. Ecological Significance of Biofilm Formation by Plant-Interacting Bacteria

Plant-associated bacteria develop a biofilm lifestyle during their interactions with plants [27][37]. Depending on whether biofilms are formed by pathogenic or beneficial individuals, the ecological outcome resulting from the interaction can be completely different. In the context of pathogenic plant-associated bacteria, the role of different components involved in biofilm formation has been studied. For instance, the biofilms formed by *Erwinia amylovora*, the causal agent of fire blight disease in different plant species of the *Rosaceae* family, and specifically the amylovoran and levan exopolysaccharides, physically blocked the vascular system of plants [43][44][45]. A mutant of *Ralstonia solanacearum*, the causal agent of bacterial wilt disease, in the *lecM* gene, which encodes a lectin, showed reduced biofilm formation in vitro and colonization of the intercellular spaces of tomato leaves and was impaired in virulence [46]. The *gumB* mutant of *Xanthomonas citri*, which produces canker disease in citrus plants, was unable to produce the polysaccharide xantan and exhibited reduced biofilm formation, survival and symptom development on lemon leaves [30]. Similarly, *Xylella fastidiosa*, which causes economically important diseases in several host plants, produced exopolysaccharides that played roles in the virulence of this bacterium, as these are required for bacterial movement within plants and plant-to plant transmission through insects [47][48].

Notably, the *Pseudomonas syringae* complex harbors most of the phytopathogens within the *Pseudomonas* genus [49][50]. In particular, the species *P. syringae* is one of the most ubiquitous bacterial participants of the phyllosphere [51]. This ubiquity, together with the fact that it can infect almost all important agricultural crops [25][52], has made it a model for the study of plant–bacteria interactions. *P. syringae* possesses a great diversity of virulence factors that engage in plant infection, as well as adaptation mechanisms that improve bacterial survival over the plant surface. Generally, *P. syringae* produces a type III secretion system (T3SS), effector proteins, motility appendages, phytotoxins, multidrug efflux pumps, extracellular polysaccharides, cell wall-degrading enzymes, and ice nucleation activity [53]. Copper- and UV radiation-resistance genes, as well as exopolysaccharide production, play fundamental roles in *P. syringae* fitness and survival [31][50][54][55][56][57][58]. In *P. syringae* pv. *syringae* (Pss), biofilm formation has been proven to influence the transition between pathogenic and epiphytic lifestyles in plants [29][31][59].

In the context of beneficial plant-associated bacteria, *Bacillus subtilis*, a Gram-positive bacterium that acts as a biocontrol agent of several plant pathogens, requires the production of extracellular matrix components involved in biofilm formation, such as those encoded by *tapA-sipW-tasA* and *epsA-O* operons, for the colonization of the plant roots and for conferring plant protection [60]. *Pseudomonas fluorescens*, an important rhizobacterium that promotes plant health and nutrition, requires biofilm formation for the colonization of plant surfaces [61]. A cellulose exopolysaccharide mutant in the *P. fluorescens* SBW25 strain was compromised in the colonization of the rhizosphere and the phyllosphere of sugar beet compared to the wild-type strain [61]. In general, the *P. fluorescens* species and some closely related species that belong to the *P. fluorescens* complex are among the most studied bacteria within soil communities, because they frequently show agricultural, biotechnological, and ecological interest, mostly due to their beneficial plant features [62]. In particular, the *P. fluorescens* and *Pseudomonas chlororaphis* species stand out because of their potential use as biocontrol agents as they frequently contribute to plant health by exerting antagonist activities against pathogens [63][64][65]. Phenotypes linked to biofilm formation have also been observed to favor bacteria–plant root interactions and biocontrol activity of *P. chlororaphis* and *Pseudomonas putida* species [66][67][68][69][70]. Usually, biocontrol agents can form biofilms, and increasing evidence strongly suggests that biofilm-forming ability should be considered in assessing their potential beneficial performance [71].

3. Main Exopolysaccharides Produced by Plant-Associated *Pseudomonas*

Among all the exopolysaccharides that are produced by plant-associated *Pseudomonas* [72], those that have been mainly studied are alginate, cellulose, and Psl (Table 1). A description of their functions in biofilm formation and architecture and their ecological significance during pathogenic and beneficial plant–bacteria interactions are listed below.

Table 1. Main exopolysaccharides produced by different *Pseudomonas* spp. strains that are involved in biofilm formation.

Strain	Clusters Encoding the Main Exopolysaccharides Described in <i>Pseudomonas</i> ¹			
	<i>alg</i>	<i>wss</i>	<i>psl</i>	<i>pel</i>
<i>P. aeruginosa</i> PAO1	+ ²	- ²	+	+
<i>P. aeruginosa</i> PA14	+	-	-	+
<i>P. syringae</i> pv. <i>syringae</i> B728a	+	-	+	-
<i>P. syringae</i> pv. <i>tomato</i> DC3000	+	+	+	-
<i>P. savastanoi</i> pv. <i>phaseolicola</i> 1448A	+	+	+	-
<i>P. syringae</i> pv. <i>syringae</i> UMAF0158	+	+	+	-
<i>P. fluorescens</i> SBW25	+	+	+	-
<i>P. fluorescens</i> Pf0-1	+	-	-	-
<i>P. fluorescens</i> F113	+	-	-	-
<i>P. chlororaphis</i> PCL1606	+	-	+	-
<i>P. chlororaphis</i> O6	+	-	+	-
<i>P. chlororaphis</i> subsp. <i>aureofaciens</i> 30–84	+	-	+	-
<i>P. putida</i> KT2440	+	- ³	-	-

¹ The *wss* operon (cellulose) of *Pseudomonas fluorescens* SBW25 and the alginate (*alg*), *psl* and *pel* operons of *Pseudomonas aeruginosa* PAO1 strains were used to perform BLASTN discontinuous megablast searches against the genome of several strains belonging to different *Pseudomonas* species. ² +, presence of the exopolysaccharide gene cluster; -, absence of the exopolysaccharide gene cluster. ³ The *wss* cluster of *Pseudomonas putida* KT2440 strain (PP2629-PP2638 genomic region) was not detected using the *wss* operon of the *P. fluorescens* SBW25 strain. However, a *wss* cluster has been previously reported to be present in this strain [73].

3.1. Alginate Exopolysaccharide

Alginate is a copolymer made of O-acetylated D-mannuronic and L-glucuronic acid residues joined by β -1,4 linkages [74]. In PAO1, the alginate polysaccharide is encoded on a twelve gene operon that corresponds to the PA3540-PA3551 genomic region [13]. During infections in cystic fibrosis (CF) patients, *P. aeruginosa* undergoes a switch into a mucoid phenotype characterized by alginate overproduction [75][76][77]. Alginate overexpression increases the resistance of *P. aeruginosa* to antimicrobial treatments, predators, and host defenses [12][78]. The high frequency in which this conversion occurs, and the protective capacities described for alginate, suggests that alginate is the main exopolysaccharide of the *P. aeruginosa* extracellular matrix. However, studies performed on nonmucoid *P. aeruginosa* strains (e.g., PAO1 and PA14), the truly predominant phenotype and the one responsible for the colonization of the lungs of CF patients [13], have shown that, although it is not critical for biofilm constitution, this polysaccharide is a component of the *P. aeruginosa* extracellular matrix and can influence its biofilm architecture [13][14][79][80].

Studies performed on alginate in some plant-associated *Pseudomonas* have revealed that this polysaccharide plays minor structural roles in their biofilms, including the bacterial phytopathogen *P. syringae* and the plant-beneficial bacteria *P. fluorescens*, *P. chlororaphis*, and *P. putida* [59][70][73][81][82]. The alginate-deficient derivative of the *P. syringae* pv. *glycinea* PG4180.muc strain formed biofilms to the same extent as the wild-type strain in flow-cell chambers [81]. However, the biofilm architecture of the PssUMAF0158 Δ *alg8* strain, which does not produce alginate, showed slightly but significantly lower surface coverage and volume than the wild-type strain [59], as was previously described in *P. aeruginosa* [80]. Alginate is overproduced in some strains of *P. syringae* upon exposure to copper bactericides, which are usually applied to reduce the disease incidence caused by some plant pathogens [32]. This could be explained because exopolysaccharide production has been generally associated with a higher tolerance against toxic compounds [2][83]. Previous works have indicated that alginate polysaccharides are involved in the pathogenic interaction of *P. syringae* with plants [29][84][85]. For instance, the alginate mutant of the *P. syringae* pv. *syringae* 3525 strain, the causal agent of bacterial brown spot on bean, is significantly impaired in the colonization of bean (host) and tomato (non-host) leaves, and although it retains the ability to generate symptoms, the symptoms are less severe than those induced by the wild-type [29]. However, these results have not been observed in other *P. syringae* strains [59][86][87][88]. For example, in *P. syringae* pv.

glycinea PG4180 strain, the causal agent of bacterial blight of soybean, the expression of the AlgT regulator protein, but not alginate production *per se*, promotes survival and symptom development in plants [88]. Similarly, the PssUMAF0158 Δ alg8 mutant strain is not altered in the induction of symptoms in tomato compared to the wild-type strain [59].

The structural functions displayed by alginate in the biofilms of plant-pathogenic *Pseudomonas* are in line with those observed in the plant-beneficial *Pseudomonas*. The alginate mutant of *P. fluorescens* SBW25 still forms biofilms in flow-cell chamber experiments, but they are thinner than those formed by the wild-type strain [82]. This result is consistent with the flow-cell chamber phenotypes of the PssUMAF0158 and PAO1 alginate mutant derivative strains [59][80]. However, the alginate mutant of the biocontrol agent *P. chlororaphis* PCL1606 (PcPCL1606 Δ alg8) forms biofilms to the same extent as the wild-type in flow-cell chamber experiments and is not impaired in initial surface attachment, showing nonsignificant differences in surface coverage and volume values with respect to the wild-type [70]. Alginate has been described as the primary polysaccharide that promotes hydration under desiccating stress in *P. putida* [89][90]. In fact, alginate slightly contributes to the biofilm architecture of *P. putida* under water-limiting conditions [90]. The functions performed by alginate polysaccharide in both *P. fluorescens* and *P. putida* strains *in vivo* seem to be more relevant than those *in vitro*. For instance, the CHA211 and CHA213M mucoid variants of the *P. fluorescens* CHA0 strain, which overproduce alginate, enhance their biofilm formation abilities on carrot roots compared to the wild-type strain [91]. The genomic region located upstream of the *algD* gene of *P. putida* KT2440 is active during the colonization of maize root, which suggests that this polysaccharide could be a fitness determinant for the rhizosphere colonization ability of this bacterium [92]. Overall, these studies indicate that alginate is not a critical component for biofilm formation *in vitro* in plant-associated *Pseudomonas* and that its role seems to be more prominent *in vivo*, facilitating colonization and providing protection against stressors.

3.2. Cellulose Exopolysaccharide

Cellulose is a polymer made of D-glucose residues joined by β -1,4 glycosidic linkages and is considered a relevant biofilm matrix molecule in many environmental *Pseudomonas* species [93][94]. Several biosynthesis and regulation mechanisms have been described for bacterial cellulose, but a common role of this component is to facilitate the establishment of efficient host-bacteria interactions [95]. Previous studies reported that several plant-associated *Pseudomonas* species can produce cellulose, including the plant-associated pathogenic bacteria *P. syringae*, *P. asplenii*, *P. marginalis*, *P. corrugate*, and *P. savastanoi* and beneficial bacteria, such as *P. fluorescens* and *P. putida* [72][93][94]. Within the *Pseudomonas* genus, *P. fluorescens* SBW25 (SBW25) is traditionally used as the model strain for the study of bacterial cellulose. In SBW25, cellulose polysaccharide is encoded on a ten-gene operon (*wssA-J*) that corresponds to the PFLU0300-PFLU0309 genomic region [96]. This exopolysaccharide is involved in the formation of floating biofilms, also called pellicles, in many strains of the species mentioned above, including the SBW25 [31][93][96][97][98][99]. The *P. aeruginosa* species does not contain the cellulose operon [72]. In particular, *P. aeruginosa* PAO1 and PA14 strains, which have been traditionally used as model strains for conducting biofilm studies within the *Pseudomonas* genus, were tested for cellulose production, but in line with *in silico* observations [72], they were not found to produce this exopolysaccharide [94]. However, the PAO1 and PA14 strains contain a seven-gene operon that encodes Pel, which is a polymer composed of partially acetylated 1–4 glycosidic linkages of N-acetylgalactosamine and N-acetylglucosamine [100]. The *pel* operon is poorly conserved among environmental *Pseudomonas* [72][100][101]. Interestingly, Pel promotes the formation of pellicle biofilms, as has also been described for cellulose [102].

Among all plant-pathogenic *Pseudomonas* that have been reported to produce cellulose, studies regarding its structural roles within biofilms and biological significance have essentially been conducted on *P. syringae*. The *P. syringae* pv. *syringae* (Pss) UMAF0158 (PssUMAF0158) strain, the causal agent of bacterial apical necrosis (BAN) on mango trees, and *P. syringae* pv. *tomato* DC3000 (PtoDC3000), responsible for bacterial speck disease on tomato plants, produce cellulose as the main exopolysaccharide of their biofilms [31][59][103]. The biofilm structures formed by PssUMAF0158 and PtoDC3000 in micro-well plates are highly similar, consisting of pellicles with wrinkles on the surface that are weakly attached to the walls of the culture vessels [31][103]. Despite the structural similarities found *in vitro*, the biological performance of cellulose seems to differ in both strains. Cellulose allows PssUMAF0158 to adhere to mango leaves, and its production intimately affects the epiphytic and pathogenic stages of this strain over the plant surface [31]. Hence, the incidence and severity of necrotic symptoms developed by PssUMAF0158 on tomato leaflets are lower in the wild-type than in cellulose mutants (Δ wssB and Δ wssE mutants) and practically nonexistent in the cellulose-overproducing strain [31]. These results, together with the fact that the highest BAN symptoms coincide with cool and wet periods [104], support the proposed lifecycle of Pss strains over the mango tree, in which biofilm formation would be mainly needed during the epiphytic phase (spring/summer) when the bacteria are more exposed to the external environment, and protection against its challenging conditions becomes crucial for survival [50]. Interestingly, the link observed between cellulose production and PssUMAF0158 transition through epiphytic and pathogenic stages over the mango plant surface has not been reported in PtoDC3000. The disease symptoms developed in tomato by the PtoDC3000 wild-type strain were not different

from those of its $\Delta wssBC$ -derived mutant [105]. Furthermore, in disagreement with what has been observed in PssUMAF0158, cellulose overproduction in PtoDC3000 does not lead to a significant impact on virulence [103]. However, the PtoDC3000 *armZ* gene mutant, which does not produce alginate and does overproduce cellulose, has a reduced virulence compared to the wild-type strain [105]. Although PssUMAF0158 and PtoDC3000 are categorized as *P. syringae* species and belong to the *P. syringae* complex, this complex is comprised of a hodgepodge that, in effect, includes many other taxonomically related species [49]. A previous study revealed that the phylogenetic relationship between *P. syringae* pv. *syringae* B728a strain, closely related to PssUMAF0158, and PtoDC3000, is not very proximate. In fact, PtoDC3000, together with other strains of the tomato pathovar, seems to form a new species *Pseudomonas tomato*, pending a deeper taxonomic analysis [49]. This evidence, together with the fact that the infection assays were performed using different tomato cultivars and inoculation approaches, could all eventually account for different results.

Regarding beneficial plant-interacting *Pseudomonas*, studies on bacterial cellulose have been mainly conducted on *P. fluorescens* and *P. putida* species. Biofilm experiments on SBW25 determined that the gradients occurring within a static microcosm immediately select for the emergence of variants that occupy different niches [106]. Among those variants, the air-liquid (A–L) interface is colonized by wrinkly spreader (WS) pellicles, an SBW25-derived mutant that overproduces cellulose compared to the wild-type equivalent [107]. In *P. putida* mt2 and its plasmid-free derivative KT2440 [108] strains, cellulose plays minor roles in biofilm formation in vitro [73][89], while two additional exopolysaccharide gene clusters, putida exopolysaccharide A (*Pea*) and putida exopolysaccharide B (*Peb*), are essential for biofilm formation in this species [73][89]. Instead, the role of cellulose exopolysaccharide in *P. putida* seems to be directed more towards conferring protection, as water-limiting conditions and increasing osmolarity highly induce cellulose expression of *P. putida* mt2 [89][109]. In addition, the cellulose mutant of *P. putida* mt2 strain accumulates significantly more reactive oxygen species (ROS) than the wild-type strain upon exposure to matrix and copper stressors [109]. During plant–bacteria interactions, the cellulose exopolysaccharide of SBW25 contributes to the ecological performance of this strain in the rhizosphere and phyllosphere of sugar beet [61]. Thus, a cellulose-defective mutant of SBW25 (SM13) was compared against the wild-type in the rhizosphere, phyllosphere, and bulk soil surrounding the rhizosphere of sugar beet seedlings, and the results showed no significant differences between the fitness of SM13 relative to the wild-type in bulk soil, but significant differences were found in the rhizosphere and phyllosphere, especially in the phyllosphere [61]. Something similar has been reported in the *P. putida* mt2 strain in which the cellulose mutant is impaired in the colonization of the maize rhizosphere during competition with the wild-type equivalent [73]. These studies indicate that, while the cellulose operon does not seem to be critical for biofilm formation under laboratory conditions in *P. fluorescens* and *P. putida*, their roles in these species seem to be more pronounced in vivo.

3.3. Psl Exopolysaccharide

The Psl polysaccharide was first described in *P. aeruginosa* [102][110][111], and its structural analysis determined that it consists of a repeating pentasaccharide subunit of D-mannose, D-glucose, and L-rhamnose in a 3:1:1 ratio [112]. In PAO1, Psl was formerly described to be encoded by the 15-gene operon *psl* (*pslA–O*), which corresponds to the PA2231–PA2245 genomic region [102][110][111]. However, later works revealed that the last three genes of the operon (*pslMNO*) constitute an independent transcriptional unit [113][114][115] and are not truly required to produce Psl [112]. Except for the case of the *P. aeruginosa* PA14 strain, which does not produce Psl due to the absence of *pslA–D* genes [102], the *psl* gene cluster is present in multiple strains of *P. aeruginosa* [72][80], where it plays key roles in their biofilm lifestyles [80]. Several studies have proven the involvement of Psl in adhesion to biotic and abiotic surfaces, biofilm architecture, motility, and protection against stressors [16][19][116][117][118][119]. Although research on Psl polysaccharides has been mostly conducted in *P. aeruginosa*, the existence of a *psl*-like gene cluster has been reported in some environmental nonaeruginosa *Pseudomonas* [59][70][72][101]. Generally, the *psl*-like gene clusters found in nonaeruginosa *Pseudomonas* either lack orthologues to *pslMNO* genes or are found scattered in the genome outside the cluster. The bacterial phytopathogen PssUMAF0158 contains a *psl*-like gene cluster that does not include orthologues to the *pslCLMNO* genes and encodes a putative acetyltransferase between the *pslJ*- and *pslK*-like genes that might perform a related function to that of acyltransferase PslL [59]. Interestingly, the *psl*-like gene cluster of PssUMAF0158 seems to be highly conserved among the plant-associated phylogroups belonging to the *P. syringae* complex [59]. The biocontrol agent PcPCL1606 also contains a *psl*-like gene cluster, which lacks the *pslLMNO* genes and encodes a putative acetyltransferase between the *pslJ*- and *pslK*-like genes, similar to that of PssUMAF0158 [70]. However, the *psl*-like gene cluster of PcPCL1606 is not present in some phylogroups of the *P. fluorescens* complex and is partially present in others, according to the strains included in a previous study [70]. It is completely absent in the *corrugata*, *jessenii*, and *koreensis* phylogroups; only present in *Pseudomonas* GM21 strain of the *mandelii* phylogroup; and is partially present within the *P. fluorescens* phylogroup. Interestingly, a *psl*-like gene cluster is found in all the strains of the *P. chlororaphis* phylogroup that have been assessed [70], which suggests that this polysaccharide could be relevant for biofilm formation in this species.

The first study regarding Psl composition in *P. aeruginosa* PAO1 determined that this polysaccharide was a galactose- and mannose-rich exopolysaccharide [120]. Support for this information came from three pieces of evidence. First, a chemical composition analysis of exopolysaccharide preparations of WFPA801, a PAO1-derived Psl-inducible strain, determined the presence of galactose, mannose, and glucose, as well as trace amounts of xylose, rhamnose, and N-acetylglucosamine. Second, staining of planktonically grown WFPA801 cells with FITC-HHA lectin, which binds to some mannosyl units, and FITC-MOA lectin, which binds to some galactosyl units, revealed green fluorescent signals on the WFPA801 surface. Ultimately, mutants of the *pslH* gene, which encodes a putative galactosyltransferase, and the *pslI* gene, which encodes a putative mannosyltransferase, were deficient in attachment, yielding a similar phenotype to that of the WFPA800 null Psl-producing strain [120]. Two years later, the structural analysis of Psl was published, indicating that it likely consisted of a pentasaccharide repeating unit of mannose, glucose, and rhamnose in approximate ratios of 3:1:1 [112]. Interestingly, galactose, which was reported as the major component of Psl in the first study [120], was not detected as a component of Psl in the structural analysis [112]. The authors stated that different growth conditions were used in both studies, which could account for some variations in composition, as described previously [121]. Therefore, there is some thought that different forms of Psl might be produced even in the same strain depending on the growth conditions. Be that as it may, mannose seems to be a key component of the Psl structure in *P. aeruginosa*. An analysis of the composition and structure of the putative Psl polysaccharide produced by PssUMAF0158 and PcPCL1606 has not yet been conducted, but some hints exist regarding the existence of a polysaccharide that resembles Psl in *P. syringae* and *P. fluorescens*. Thus, it was reported that, in addition to alginate and levan, *P. syringae* PG4180 produced a third exopolysaccharide (EPS) that consisted of a fibrous structure in its biofilms and bound to *Naja mossambica* lectin (NML) [81]. Interestingly, the monosaccharide specificity of NML is mannose [122]. Furthermore, two *P. fluorescens* strains isolated from rotted bell pepper, PF-05-2 and PM-LB-1, produced a novel exopolysaccharide composed of mannose, rhamnose, and glucose (1:1:1 molar ratio) substituted with pyruvate and acetate [123]. The biofilm formed by the PcPCL1606 wild-type strain but not its Psl-like-derived mutant, contains a polymer that binds to banana lectin, which also binds to mannose residues [70][124].

The Δ *pslAB* mutant of PAO1 is severely attenuated in biofilm initiation and biofilm development in flow-cell chamber experiments [110][111]. Interestingly, similar results were observed in the biocontrol strain PcPCL1606, in which the Δ *pslE* mutant was severely affected in early surface attachment and development of a mature biofilm architecture compared to the wild-type strain [70]. The biofilms developed by PAO1 and PcPCL1606 wild-type strains showed an intricate architecture in flow-cell chambers, which consisted of a multilayer of cells that covered the chamber surfaces [70][110][111]. However, the biofilm phenotype of the PAO1 Δ *pslAB* and PcPCL1606 Δ *pslE* mutant strains consisted of a monolayer of loosely aggregated cells, which suggests that this exopolysaccharide could also be important for cell-to-cell interactions [70][110][111]. Similarly, the Psl-like polysaccharide of the phytopathogen PssUMAF0158 is also involved in biofilm architecture [59]. Compared to the more developed biofilm of wild-type PssUMAF0158, the PssUMAF0158 Δ *pslE* biofilms consisted of scattered cell aggregates across the flow-cell chamber surface [59]. These cell aggregates were disrupted in the double mutant Δ *wssE*,*pslE* strain, which did not produce both cellulose and Psl-like polysaccharides [59]. Curiously, this phenotype was also observed in some *P. aeruginosa* strains, where the cell aggregates formed by their derived Δ *psl* mutants were disrupted in the Δ *psl* Δ *pel* double mutants, affected in both Psl and Pel polysaccharide production [116]. The fact that cellulose and Pel polysaccharides are both involved in the formation of pellicle biofilms [101], and that the cell aggregates formed by these *Pseudomonas* Δ *psl* strains are disrupted when either cellulose or Pel is not produced, indicates that both polysaccharides could play redundant structural roles within biofilms, as has been previously suggested [59][101]. Indeed, it is not common to find both genomic regions encoding cellulose and Pel in the same *Pseudomonas* strain. Thus, just 12 out of 600 *Pseudomonas* genomes (2%) that have been analyzed in a recent study [72]—which belong to four different groups: *P. asplenii*, *P. fluorescens*, *P. fragi* and *P. oryzae*—possess both clusters (Table 2), although whether they are functional remains unknown. With these recent data, the identity and coverage of both clusters have been analyzed in these 12 *Pseudomonas* spp. strains using the *wss* operon of SBW25 and *pel* operon of PAO1 as references.

Table 2. *Pseudomonas* spp. strains obtained from Blanco–Romero et al., (2020) that have been reported to contain the *wss* and *pel* clusters.

Strain	<i>wss</i> Cluster ¹		<i>pel</i> Cluster ¹	
	Identity (%)	Coverage (%)	Identity (%)	Coverage (%)
<i>P. agarici</i> NCPPB 2472	71.03	82	69.68	93
<i>P. azotoformans</i> F77	82.58	100	70.63	89

Strain	wss Cluster ¹		pel Cluster ¹	
<i>P. azotoformans</i> LMG_21611	83.72	99	70.50	91
<i>P. extremorientalis</i> LMG 19695	89.82	99	70.59	92
<i>P. lundensis</i> AU1044	71.31	11 ²	72.32	91
<i>P. lurida</i> L228	83.17	100	71.37	85
<i>P. lurida</i> MYb11	82.99	100	71.48	85
<i>P. oryzae</i> USDA-ARS-USMARC-56511	68.70	53	71.33	98
<i>P. oryzae</i> FDAARGOS_657	70.45	57	71.50	98
<i>P. psychrotolerans</i> PRS08-11306	70.40	58	71.66	98
<i>P. psychrotolerans</i> CS51	70.11	50	74.27	94
<i>P. trivialis</i> IHBB745	91.38	99	73.28	91

¹ The wss operon (cellulose) of *P. fluorescens* SBW25 and the pel operon of *P. aeruginosa* PAO1 were used to perform BLASTN discontinuous megablast searches against the genome of the 12 *Pseudomonas* strains that harbor both exopolysaccharide gene clusters. ² The low coverage obtained in *P. lundensis* AU1044 with regard to the SBW25 cellulose cluster could be due to different approaches performed to assess the presence of exopolysaccharide clusters.

Swarming motility and biosurfactant synthesis are coordinated with Psl production in *P. aeruginosa*, as the PAO1 Psl-deficient strain exhibited a hyperswarming phenotype due to an increase in rhamnolipid production, and vice versa [118]. Curiously, the link found between biofilm formation, rhamnolipid production, and motility in the bacterial phytopathogen PssUMAF0158 seems opposite to that described in *P. aeruginosa*. Therefore, the PssUMAF0158 Δ pslE mutant is impaired in swarming motility compared to the wild-type strain, and this impairment could be due to a reduction in surfactant production, as the *rhlA* gene involved in rhamnolipid precursor synthesis is downregulated in the mutant compared to the wild-type strain [59].

The PAO1 Δ pslAB mutant is deficient in biofilm initiation due to its reduced ability to interact with biotic and abiotic surfaces [16][110][111]. In line with these data, the recently described Psl-like exopolysaccharides of the bacterial phytopathogen PssUMAF0158 and biocontrol agent PcPCL1606 have also been reported to be involved in early surface interactions. The PssUMAF0158 Δ pslE mutant is impaired in early adhesion to mango leaves [59] and the PcPCL1606 Δ pslE mutant was impaired in early surface attachment to polystyrene micro-well plates and avocado root surfaces [70]. Moreover, biofilm formation by PssUMAF0158 and PcPCL1606 strains through Psl-like exopolysaccharide biosynthesis also contributes to the lifestyles displayed by these bacteria during interaction with their plant host [59][70]. The inability to produce some extracellular matrix components, such as cellulose and Psl-like exopolysaccharides, seems to predispose PssUMAF0158 to the pathogenic lifestyle, as the mutants impaired in the production of these exopolysaccharides are significantly more virulent than the wild-type [31][59]. Consequently, the biofilm lifestyle of PssUMAF0158 could predominate during the epiphytic phase, as has been previously suggested [50]. Furthermore, the Psl-like exopolysaccharide of PcPCL1606 contributes to the biocontrol activity of this bacterium against white rot root disease caused by *Rosellinia necatrix* in avocado plants [70]. Thus, PcPCL1606 Δ pslE is severely compromised in disease suppression, probably because early attachment and biofilm impairments could lead to inefficient colonization of roots, which is a prerequisite for efficient disease control [70][125][126][127]. The presence of a psl-like cluster in some environmental pseudomonads with different lifestyles suggests that this polysaccharide might constitute a general feature of biofilm formation of these bacteria, providing different functions depending on the genetic context and niche of production.

References

- Costerton, J.W.; Lewandowski, Z. Microbial biofilms. Annu. Rev. Microbiol. 1995, 49, 711–745.
- Costerton, J.W. Introduction to biofilm. Int. J. Antimicrob. Agents 1999, 11, 217–221.
- Høiby, N.; Bjarnsholt, T.; Givskov, M.; Molin, S.; Ciofu, O. Antibiotic resistance of bacterial biofilms. Int. J. Antimicrob. Agents 2010, 35, 322–332.

4. Marshall, K.C. Planktonic versus sessile life of prokaryotes. In *The Prokaryotes*, 3rd ed.; Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E., Eds.; Springer: New York, NY, USA, 2006; Volume 2, pp. 3–15.
5. Flemming, H.C.; Wuertz, S. Bacteria and archaea on Earth and their abundance in biofilms. *Nat. Rev. Microbiol.* 2019, 17, 247–260.
6. Bar-On, Y.M.; Milo, R. Towards a quantitative view of the global ubiquity of biofilms. *Nat. Rev. Microbiol.* 2019, 17, 199–200.
7. Jefferson, K.K. What drives bacteria to produce a biofilm? *FEMS Microbiol. Lett.* 2004, 136, 163–173.
8. Yin, W.; Wang, Y.; Liu, L.; He, J. Biofilms: The microbial "protective clothing" in extreme environments. *Int. J. Mol. Sci.* 2019, 20, 3423.
9. Silby, M.W.; Winstanley, C.; Godfrey, S.A.; Levy, S.B.; Jackson, R.W. *Pseudomonas* genomes: Diverse and adaptable. *FEMS Microbiol. Rev.* 2011, 35, 652–680.
10. Diggle, S.P.; Whiteley, M. Microbe profile: *Pseudomonas aeruginosa*: Opportunistic pathogen and lab rat. *Microbiology* 2020, 166, 30–33.
11. Franklin, M.J.; Nivens, D.E.; Weadge, J.T.; Howell, L. Biosynthesis of the *Pseudomonas aeruginosa* extracellular polysaccharides, alginate, Pel and Psl. *Front. Microbiol.* 2011, 2, 167.
12. Hentzer, M.; Teitzel, G.M.; Balzer, G.J.; Heydorn, A.; Molin, S.; Givskov, M.; Parsek, M.R. Alginate overproduction affects *Pseudomonas aeruginosa* biofilm structure and function. *J. Bacteriol.* 2001, 183, 5395–5401.
13. Wozniak, D.J.; Wyckoff, T.J.O.; Starkey, M.; Keyser, R.; Azadi, P.; O'Toole, G.A.; Parsek, M.R. Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 *Pseudomonas aeruginosa* biofilms. *Proc. Natl. Acad. Sci. USA* 2003, 100, 7907–7912.
14. Stapper, A.P.; Narasimhan, G.; Ohman, D.E.; Barakat, J.; Hentzer, M.; Molin, S.; Kharazmi, A.; Høiby, N.; Mathee, K. Alginate production affects *Pseudomonas aeruginosa* biofilm development and architecture, but is not essential for biofilm formation. *J. Med. Microbiol.* 2004, 53, 679–690.
15. Leid, J.G.; Willson, C.J.; Shirliff, M.E.; Hassett, D.J.; Parsek, M.R.; Jeffers, A.K. The exopolysaccharide alginate protects *Pseudomonas aeruginosa* biofilm bacteria from IFN- γ -mediated macrophage killing. *J. Immunol.* 2005, 175, 7512–7518.
16. Ma, L.; Jackson, K.D.; Landry, R.M.; Parsek, M.R.; Wozniak, D.J. Analysis of *Pseudomonas aeruginosa* conditional Psl variants reveals roles for the Psl polysaccharide in adhesion and maintaining biofilm structure postattachment. *J. Bacteriol.* 2006, 188, 8213–8221.
17. Colvin, K.M.; Gordon, V.D.; Murakami, K.; Borlee, B.R.; Wozniak, D.J.; Wong, G.C.; Parsek, M.R. The Pel polysaccharide can serve a structural and protective role in the biofilm matrix of *Pseudomonas aeruginosa*. *PLoS Pathog.* 2011, 7, e1001264.
18. Ma, L.; Wang, S.; Wang, D.; Parsek, M.R.; Wozniak, D.J. The roles of biofilm matrix polysaccharide Psl in mucoid *Pseudomonas aeruginosa* biofilms. *FEMS Immunol. Med. Microbiol.* 2012, 65, 377–380.
19. Billings, N.; Millan, M.R.; Caldara, M.; Rusconi, R.; Tarasova, Y.; Stocker, R.; Ribbeck, K. The extracellular matrix component Psl provides fast-acting antibiotic defense in *Pseudomonas aeruginosa* biofilms. *PLoS Pathog.* 2013, 9, e1003526.
20. Ciofu, O.; Tolker-Nielsen, T. Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents-how *P. aeruginosa* can escape antibiotics. *Front. Microbiol.* 2019, 10, 913.
21. Elrod, R.P.; Braun, A.C. *Pseudomonas aeruginosa*: Its role as a plant pathogen. *J. Bacteriol.* 1942, 44, 633–645.
22. Walker, T.S.; Bais, H.P.; Déziel, E.; Schweizer, H.P.; Rahme, L.G.; Fall, R.; Vivanco, J.M. *Pseudomonas aeruginosa*-plant root interactions. Pathogenicity, biofilm formation, and root exudation. *Plant Physiol.* 2004, 134, 320–331.
23. Hirano, S.S.; Upper, C.D. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*-a pathogen, ice nucleus, and epiphyte. *Microbiol. Mol. Biol. Rev.* 2000, 64, 624–653.
24. Lugtenberg, B.J.J.; Dekkers, L.; Bloemberg, G.V. Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annu. Rev. Phytopathol.* 2001, 39, 461–490.
25. Kennelly, M.M.; Cazorla, F.M.; de Vicente, A.; Ramos, C.; Sundin, G.W. *Pseudomonas syringae* diseases of fruit trees: Progress toward understanding and control. *Plant Dis.* 2007, 91, 4–17.
26. Mercado-Blanco, J.; Bakker, P.A.H.M. Interactions between plants and beneficial *Pseudomonas* spp.: Exploiting bacterial traits for crop protection. *Antonie Leeuwenhoek* 2007, 92, 367–389.
27. Danhorn, T.; Fuqua, C. Biofilm formation by plant-associated bacteria. *Annu. Rev. Microbiol.* 2007, 61, 401–422.

28. Leveau, J.H. A brief from the leaf: Latest research to inform our understanding of the phyllosphere microbiome. *Curr. Opin. Microbiol.* 2019, 49, 41–49.
29. Yu, J.; Peñaloza-Vázquez, A.; Chakrabarty, A.M.; Bender, C.L. Involvement of the exopolysaccharide alginate in the virulence and epiphytic fitness of *Pseudomonas syringae* pv. *syringae*. *Mol. Microbiol.* 1999, 33, 712–720.
30. Rigano, L.A.; Siciliano, F.; Enrique, R.; Sendín, L.; Filippone, P.; Torres, P.S.; Qüesta, J.; Dow, J.M.; Castagnaro, A.P.; Vojnov, A.A.; et al. Biofilm formation, epiphytic fitness, and canker development in *Xanthomonas axonopodis* pv. *citri*. *Mol. Plant Microbe Interact.* 2007, 20, 1222–1230.
31. Arrebola, E.; Carrión, V.J.; Gutiérrez-Barranquero, J.A.; Rodríguez-Palenzuela, P.; Cazorla, F.M.; de Vicente, A. Cellulose production in *Pseudomonas syringae* pv. *syringae*: A compromise between epiphytic and pathogenic lifestyles. *FEMS Microbiol. Ecol.* 2015, 91, 1–12.
32. Kidambi, S.P.; Sundin, G.W.; Palmer, D.A.; Chakrabarty, A.M.; Bender, C.L. Copper as a signal for alginate synthesis in *Pseudomonas syringae* pv. *syringae*. *Microbiology* 1995, 61, 2172–2179.
33. Ophir, T.; Gutnick, D.L. A role for exopolysaccharides in the protection of microorganisms from desiccation. *Appl. Environ. Microbiol.* 1994, 60, 740–745.
34. Dong, C.J.; Wang, L.L.; Li, Q.; Shang, Q.M. Bacterial communities in the rhizosphere, phyllosphere and endosphere of tomato plants. *PLoS ONE* 2019, 14, e0223847.
35. Fageria, N.K.; Stone, L.F. Physical, chemical, and biological changes in the rhizosphere and nutrient availability. *J. Plant Nutr.* 2006, 29, 1327–1356.
36. Thies, J.E.; Grossman, J.M. The soil habitat and soil ecology. In *Biological Approaches to Sustainable Soil Systems*, 1st ed.; Uphoff, N., Ball, A.S., Fernandes, E., Herren, H., Husson, O., Laing, M., Palm, C., Pretty, J., et al., Eds.; CRC Press: Boca Raton, FL, USA, 2006; Chapter 5; p. 20.
37. Bogino, P.C.; Oliva, M.; Sorroche, F.G.; Giordano, W. The role of bacterial biofilms and surface components in plant-bacterial associations. *Int. J. Mol. Sci.* 2013, 14, 15838–15859.
38. Rudrappa, T.; Biedrzycki, M.L.; Bais, H.P. Causes and consequences of plant-associated biofilms. *FEMS Microbiol. Ecol.* 2008, 64, 153–166.
39. Davey, M.E.; O'Toole, G.A. Microbial biofilms: From ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.* 2000, 64, 847–867.
40. Lynch, J.M.; de Leij, F. Rhizosphere. In *eLS; Key Concepts*; John Wiley & Sons, Ltd.: Chichester, UK, 2012.
41. Sandhya, V.; Ali, S.Z. The production of exopolysaccharide by *Pseudomonas putida* GAP-P45 under various abiotic stress conditions and its role in soil aggregation. *Microbiology* 2015, 84, 512–519.
42. Costa, O.Y.A.; Raaijmakers, J.M.; Kuramae, E.E. Microbial extracellular polymeric substances: Ecological function and impact on soil aggregation. *Front. Microbiol.* 2018, 9, 1636.
43. Sjulín, T.M.; Beer, S.V. Mechanism of wilt induction by amylovorin in *Cotoneaster* shoots and its relation to wilting of shoots infected by *Erwinia amylovora*. *Phytopathology* 1978, 68, 89.
44. Koczan, J.M.; McGrath, M.J.; Zhao, Y.; Sundin, G.W. Contribution of *Erwinia amylovora* exopolysaccharides amylovan and levan to biofilm formation: Implications in pathogenicity. *Phytopathology* 2009, 99, 1237–1244.
45. Koczan, J.M.; Lenneman, B.R.; McGrath, M.J.; Sundin, G.W. Cell surface attachment structures contribute to biofilm formation and xylem colonization by *Erwinia amylovora*. *Appl. Environ. Microbiol.* 2011, 77, 7031–7039.
46. Mori, Y.; Inoue, K.; Ikeda, K.; Nakayashiki, H.; Higashimoto, C.; Ohnishi, K.; Kiba, A.; Hikichi, Y. The vascular plant-pathogenic bacterium *Ralstonia solanacearum* produces biofilms required for its virulence on the surfaces of tomato cells adjacent to intercellular spaces. *Mol. Plant Pathol.* 2016, 17, 890–902.
47. Marques, L.L.R.; Ceri, H.; Manfio, G.P.; Reid, D.M.; Olson, M.E. Characterization of biofilm formation by *Xylella fastidiosa* in vitro. *Plant Dis.* 2020, 86, 633–638.
48. Killiny, N.; Martinez, R.H.; Dumenyo, C.K.; Cooksey, D.A.; Almeida, R.P. The exopolysaccharide of *Xylella fastidiosa* is essential for biofilm formation, plant virulence, and vector transmission. *Mol. Plant Microbe Interact.* 2013, 26, 1044–1053.
49. Gomila, M.; Busquets, A.; Mulet, M.; García-Valdés, E.; Lalucat, J. Clarification of taxonomic status within the *Pseudomonas syringae* species group based on a phylogenomic analysis. *Front. Microbiol.* 2017, 8, 1–13.
50. Gutiérrez-Barranquero, J.A.; Cazorla, F.M.; de Vicente, A. *Pseudomonas syringae* pv. *syringae* associated with mango trees, a particular pathogen within the “hodgepodge” of the *Pseudomonas syringae* complex. *Front. Plant Sci.* 2019, 10, 570.

51. Lindow, S.E.; Brandl, M.T. Microbiology of the phyllosphere. *Appl Environ. Microbiol.* 2003, 69, 1875–1883.
52. Xin, X.F.; Kvitko, B.; He, S.Y. *Pseudomonas syringae*: What it takes to be a pathogen. *Nat. Rev. Microbiol.* 2018, 16, 139–148.
53. Ichinose, Y.; Taguchi, F.; Mukaiyama, T. Pathogenicity and virulence factors of *Pseudomonas syringae*. *J. Gen. Plant Pathol.* 2013, 79, 285–296.
54. Sundin, G.W.; Kidambi, S.P.; Ullrich, M.; Bender, C.L. Resistance to ultraviolet light in *Pseudomonas syringae*: Sequence and functional analysis of the plasmid-encoded *rulAB* genes. *Gene* 1996, 177, 77–81.
55. Cazorla, F.M.; Arrebola, E.; Sesma, A.; Pérez-García, A.; Codina, J.C.; Murillo, J.; de Vicente, A. Copper resistance in *Pseudomonas syringae* strains isolated from mango is encoded mainly by plasmids. *Phytopathology* 2002, 92, 909–916.
56. Gutiérrez-Barranquero, J.A.; de Vicente, A.; Carrión, V.J.; Sundin, G.W.; Cazorla, F.M. Recruitment and rearrangement of three different genetic determinants into a conjugative plasmid increase copper resistance in *Pseudomonas syringae*. *Appl. Environ. Microbiol.* 2013, 79, 1028–1033.
57. Gutiérrez-Barranquero, J.A.; Cazorla, F.M.; de Vicente, A.; Sundin, G.W. Complete sequence and comparative genomic analysis of eight native *Pseudomonas syringae* plasmids belonging to the pPT23A family. *BMC Genom.* 2017, 18, 365.
58. Aprile, F.; Heredia-Ponce, Z.; Cazorla, F.M.; de Vicente, A.; Gutiérrez-Barranquero, J.A. A large Tn7-like transposon confers hyper-resistance to copper in *Pseudomonas syringae* pv. *syringae*. *Appl. Environ. Microbiol.* 2021, 87, e02528-20.
59. Heredia-Ponce, Z.; Gutiérrez-Barranquero, J.A.; Purtschert-Montenegro, G.; Eberl, L.; Cazorla, F.M.; de Vicente, A. Biological role of EPS from *Pseudomonas syringae* pv. *syringae* UMAF0158 extracellular matrix, focusing on a Psl-like polysaccharide. *NPJ Biofilms Microbiomes* 2020, 6, 37.
60. Chen, Y.; Yan, F.; Chai, Y.; Liu, H.; Kolter, R.; Losick, R.; Guo, J.H. Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. *Environ. Microbiol.* 2013, 15, 848–864.
61. Gal, M.; Preston, G.M.; Massey, R.C.; Spiers, A.J.; Rainey, P.B. Genes encoding a cellulosic polymer contribute toward the ecological success of *Pseudomonas fluorescens* SBW25 on plant surfaces. *Mol. Ecol.* 2003, 12, 3109–3121.
62. Garrido-Sanz, D.; Arrebola, E.; Martínez-Granero, F.; García-Méndez, S.; Muriel, C.; Blanco-Romero, E.; Martín, M.; Rivila, R.; Redondo-Nieto, M. Classification of isolates from the *Pseudomonas fluorescens* complex into phylogenomic groups based in group-specific markers. *Front. Microbiol.* 2017, 8, 413.
63. Ganeshan, G.; Kumar, A.M. *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. *J. Plant Interact.* 2005, 1, 123–134.
64. Cazorla, F.M.; Duckett, S.B.; Bergström, E.T.; Noreen, S.; Odijk, R.; Lugtenberg, B.J.J.; Thomas-Oates, J.E.; Bloembergen, G.V. Biocontrol of avocado dematophora root rot by antagonistic *Pseudomonas fluorescens* PCL1606 correlates with the production of 2-hexyl 5-propyl resorcinol. *Mol. Plant Microbe Interact.* 2006, 19, 418–428.
65. Anderson, A.J.; Kim, Y.C. Insights into plant-beneficial traits of probiotic *Pseudomonas chlororaphis* isolates. *J. Med. Microbiol.* 2020, 69, 361–371.
66. Calderón, C.E.; Pérez-García, A.; de Vicente, A.; Cazorla, F.M. The *dar* genes of *Pseudomonas chlororaphis* PCL1606 are crucial for biocontrol activity via production of the antifungal compound 2-hexyl, 5-propyl resorcinol. *Mol. Plant-Microbe Interact.* 2013, 26, 554–565.
67. Calderón, C.E.; de Vicente, A.; Cazorla, F.M. Role of 2-hexyl, 5-propyl resorcinol production by *Pseudomonas chlororaphis* PCL1606 in the multitrophic interactions in the avocado rhizosphere during the biocontrol process. *FEMS Microbiol. Ecol.* 2014, 89, 20–31.
68. Calderón, C.E.; Tienda, S.; Heredia-Ponce, Z.; Arrebola, E.; Cárcamo-Oyarce, G.; Eberl, L.; Cazorla, F.M. The compound 2-hexyl, 5-propyl resorcinol has a key role in biofilm formation by the biocontrol rhizobacterium *Pseudomonas chlororaphis* PCL1606. *Front. Microbiol.* 2019, 10, 369.
69. Sun, D.; Zhuo, T.; Hu, X.; Fan, X.; Zou, H. Identification of a *Pseudomonas putida* as biocontrol agent for tomato bacterial wilt disease. *Biol. Control* 2017, 114, 45–50.
70. Heredia-Ponce, Z.M.; Gutiérrez-Barranquero, J.A.; Purtschert-Montenegro, G.; Eberl, L.; de Vicente, A.; Cazorla, F.M. Role of extracellular matrix components in the formation of biofilms and their contribution to the biocontrol activity of *Pseudomonas chlororaphis* PCL1606. *Environ. Microbiol.* 2020.

71. Pandin, C.; Le Coq, D.; Canette, A.; Aymerich, S.; Briandet, R. Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? *Microb. Biotechnol.* 2017, 10, 719–734.
72. Blanco-Romero, E.; Garrido-Sanz, D.; Rivilla, R.; Redondo-Nieto, M.; Martín, M. In silico characterization and phylogenetic distribution of extracellular matrix components in the model rhizobacteria *Pseudomonas fluorescens* F113 and other *Pseudomonads*. *Microorganisms* 2020, 8, 1740.
73. Nilsson, M.; Chiang, W.C.; Fazli, M.; Gjermansen, M.; Givskov, M.; Tolker-Nielsen, T. Influence of putative exopolysaccharide genes on *Pseudomonas putida* KT2440 biofilm stability. *Environ. Microbiol.* 2011, 13, 1357–1369.
74. Evans, L.R.; Linker, A. Production and characterization of the slime polysaccharide of *Pseudomonas aeruginosa*. *J. Bacteriol.* 1973, 116, 915–924.
75. Fegan, M.; Francis, P.; Hayward, A.C.; Davis, G.H.; Fuerst, J.A. Phenotypic conversion of *Pseudomonas aeruginosa* in cystic fibrosis. *J. Clin. Microbiol.* 1990, 28, 1143–1146.
76. Terry, J.M.; Piña, S.E.; Mattingly, S.J. Environmental conditions which influence mucoid conversion *Pseudomonas aeruginosa* PAO1. *Infect. Immun.* 1991, 59, 471–477.
77. Lyczak, J.B.; Cannon, C.L.; Pier, G.B. Lung infections associated with cystic fibrosis. *Clin. Microbiol. Rev.* 2002, 15, 194–222.
78. Simpson, J.A.; Smith, S.E.; Dean, R.T. Alginate inhibition of the uptake of *Pseudomonas aeruginosa* by macrophages. *J. Gen. Microbiol.* 1988, 134, 29–36.
79. McIntyre-Smith, A.; Schneiderman, J.; Zhou, K. Alginate does not appear to be essential for biofilm production by PAO1 *Pseudomonas aeruginosa*. *J. Exp. Microbiol. Immunol.* 2010, 14, 63–68.
80. Ghafoor, A.; Hay, I.D.; Rehm, B.H.A. Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture. *Appl. Environ. Microbiol.* 2011, 77, 5238–5246.
81. Laue, H.; Schenk, A.; Li, H.; Lambertsen, L.; Neu, T.R.; Molin, S.; Ullrich, M.S. Contribution of alginate and levan production to biofilm formation by *Pseudomonas syringae*. *Microbiology* 2006, 152, 2909–2918.
82. Noirot-Gros, M.F.; Forrester, S.; Malato, G.; Larsen, P.E.; Noirot, P. CRISPR interference to interrogate genes that control biofilm formation in *Pseudomonas fluorescens*. *Sci. Rep.* 2019, 9, 15954.
83. Goltermann, L.; Tolker-Nielsen, T. Importance of the exopolysaccharide matrix in antimicrobial tolerance of *Pseudomonas aeruginosa* aggregates. *Antimicrob. Agents Chemother.* 2017, 61, 1–7.
84. Rudolph, K.W.E.; Gross, M.; Ebrahim-Nesbat, F.; Nöllenburg, M.; Zomorodian, A.; Wydra, K.; Neugebauer, M.; Hettwer, U.; El-Shouny, W.; Sonnerberg, B.; et al. The role of extracellular polysaccharides as virulence factors for phytopathogenic pseudomonads and xanthomonads. In *Molecular Mechanisms of Bacterial Virulence*; Kado, C.I., Crosa, J.H., Eds.; Springer: Dordrech, The Netherlands, 1994; Volume 3, pp. 357–358.
85. Fett, W.F.; Dunn, M.F. Exopolysaccharides produced by phytopathogenic *Pseudomonas syringae* pathovars in infected leaves of susceptible hosts. *Plant Physiol.* 1989, 89, 5–9.
86. Peñaloza-Vázquez, A.; Kidambi, S.P.; Chakrabarty, A.M.; Bender, C.L. Characterization of the alginate biosynthetic gene cluster in *Pseudomonas syringae* pv. *syringae*. *Microbiology* 1997, 179, 4464–4472.
87. Willis, D.K.; Holmstadt, J.J.; Kinscherf, T.G. Genetic evidence that loss of virulence associated with *gacS* or *gacA* mutations in *Pseudomonas syringae* B728a does not result from effects on alginate production. *Appl. Environ. Microbiol.* 2001, 67, 1400–1403.
88. Schenk, A.; Weingart, H.; Ullrich, M. The alternative sigma factor AlgT, but not alginate synthesis, promotes in planta multiplication of *Pseudomonas syringae* pv. *glycinea*. *Microbiology* 2008, 154, 413–421.
89. Nielsen, L.; Li, X.; Halverson, L.J. Cell-cell and cell-surface interactions mediated by cellulose and a novel exopolysaccharide contribute to *Pseudomonas putida* biofilm formation and fitness under water-limiting conditions. *Environ. Microbiol.* 2011, 13, 1342–1356.
90. Chang, W.S.; van de Mortel, M.; Nielsen, L.; Nino de Guzman, G.; Li, X.; Halverson, L.J. Alginate production by *Pseudomonas putida* creates a hydrated microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions. *J. Bacteriol.* 2007, 189, 8290–8299.
91. Bianciotto, V.; Andreotti, S.; Balestrini, R.; Bonfante, P.; Perotto, S. Mucoid mutants of the biocontrol strain *Pseudomonas fluorescens* CHA0 show increased ability in biofilm formation on mycorrhizal and nonmycorrhizal carrot roots. *Mol. Plant Microbe Interact.* 2001, 14, 255–260.
92. Ramos-González, M.I.; Campos, M.J.; Ramos, J.L. Analysis of *Pseudomonas putida* KT2440 gene expression in the maize rhizosphere: In vivo expression technology capture and identification of root-activated promoters. *J. Bacteriol.* 2005, 187, 5504.

93. Ude, S.; Arnold, D.L.; Moon, C.D.; Timms-Wilson, T.; Spiers, A.J. Biofilm formation and cellulose expression among diverse environmental *Pseudomonas* isolates. *Environ. Microbiol.* 2006, 8, 1997–2011.
94. Spiers, A.J.; Deeni, Y.Y.; Folorunso, A.O.; Koza, A.; Moshynets, O.; Zawadzki, K. Cellulose expression in *Pseudomonas fluorescens* SBW25 and other environmental pseudomonads. In *Cellulose-Medical, Pharmaceutical and Electronic Applications*; van de Ven, T., Godbout, L., Eds.; IntechOpen: Rijeka, Croatia, 2013; Chapter 1.
95. Augimeri, R.V.; Varley, A.J.; Strap, J.L. Establishing a role for bacterial cellulose in environmental interactions: Lessons learned from diverse biofilm-producing Proteobacteria. *Front. Microbiol.* 2015, 6, 1282.
96. Spiers, A.J.; Bohannon, J.; Gehrig, S.M.; Rainey, P.B. Biofilm formation at the air-liquid interface by the *Pseudomonas fluorescens* SBW25 wrinkly spreader requires an acetylated form of cellulose. *Mol. Microbiol.* 2003, 50, 15–27.
97. Armitano, J.; Méjean, V.; Jourlin-Castelli, C. Floating biofilm in Gram-negative bacteria. *Environ. Microbiol. Rep.* 2014, 6, 534–544.
98. Ardré, M.; Dufour, D.; Rainey, P.B. Causes and biophysical consequences of cellulose production by *Pseudomonas fluorescens* SBW25 at the air-liquid interface. *J. Bacteriol.* 2019, 201, e00110-19.
99. Farias, G.A.; Olmedilla, A.; Gallegos, M.T. Visualization and characterization of *Pseudomonas syringae* pv. tomato DC3000 pellicles. *Microb. Biotechnol.* 2019, 12, 688–702.
100. Jennings, L.K.; Storek, K.M.; Ledvina, H.E.; Coulon, C.; Marmont, L.S.; Sadovskaya, I.; Secor, P.R.; Tseng, B.S.; Scian, M.; Filloux, A.; et al. Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the *Pseudomonas aeruginosa* biofilm matrix. *Proc. Natl. Acad. Sci. USA* 2015, 112, 11353–11358.
101. Mann, E.E.; Wozniak, D.J. *Pseudomonas* biofilm matrix composition and niche biology. *FEMS Microbiol.* 2012, 36, 893–916.
102. Friedman, L.; Kolter, R. Two genetic loci produce distinct carbohydrate-rich structural components of the *Pseudomonas aeruginosa* biofilm matrix. *J. Bacteriol.* 2004, 186, 4457–4465.
103. Pérez-Mendoza, D.; Aragón, I.M.; Prada-Ramírez, H.A.; Romero-Jiménez, L.; Ramos, C.; Gallegos, M.T.; Sanjuán, J. Responses to elevated c-di-GMP levels in mutualistic and pathogenic plant-interacting bacteria. *PLoS ONE* 2014, 9, e91645.
104. Cazorla, F.M.; Torés, J.A.; Olalla, L.; Pérez-García, A.; Farré, J.M.; de Vicente, A. Bacterial apical necrosis of mango in Southern Spain: A disease caused by *Pseudomonas syringae* pv. *syringae*. *Phytopathology* 1998, 88, 614–620.
105. Prada-Ramírez, H.A.; Pérez-Mendoza, D.; Felipe, A.; Martínez-Granero, F.; Rivilla, R.; Sanjuán, J.; Gallegos, M.T. AmrZ regulates cellulose production in *Pseudomonas syringae* pv. tomato DC3000. *Mol. Microbiol.* 2016, 99, 960–977.
106. Rainey, P.B.; Travisano, M. Adaptive radiation in a heterogeneous environment. *Nature* 1998, 394, 69–72.
107. Spiers, A.J.; Kahn, S.G.; Bohannon, J.; Travisano, M.; Rainey, P.B. Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. *Genetics* 2002, 161, 33–46.
108. Nakazawa, T. Travels of a *Pseudomonas*, from Japan around the world. *Environ. Microbiol.* 2002, 4, 782–786.
109. Svenningsen, N.B.; Martínez-García, E.; Nicolaisen, M.H.; de Lorenzo, V.; Nybroe, O. The biofilm matrix polysaccharides cellulose and alginate both protect *Pseudomonas putida* mt-2 against reactive oxygen species generated under matrix stress and copper exposure. *Microbiology* 2018, 164, 883–888.
110. Jackson, K.D.; Starkey, M.; Kremer, S.; Parsek, M.R.; Wozniak, D.J. Identification of psl, a locus encoding a potential exopolysaccharide that is essential for *Pseudomonas aeruginosa* PAO1 biofilm formation. *J. Bacteriol.* 2004, 186, 4466–4475.
111. Matsukawa, M.; Greenberg, E.P. Putative exopolysaccharide synthesis genes influence *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.* 2004, 186, 4449–4456.
112. Byrd, M.S.; Sadovskaya, I.; Vinogradov, E.; Lu, H.; Sprinkle, A.B.; Richardson, S.H.; Ma, L.; Ralston, B.; Parsek, M.R.; Anderson, E.M.; et al. Genetic and biochemical analyses of the *Pseudomonas aeruginosa* Psl exopolysaccharide reveal overlapping roles for polysaccharide synthesis enzymes in Psl and LPS production. *Mol. Microbiol.* 2009, 73, 622–638.
113. Goodman, A.L.; Kulasekara, B.; Rietsch, A.; Boyd, D.; Smith, R.S.; Lory, S. A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. *Dev. Cell* 2004, 7, 745–754.
114. Hickman, J.W.; Tifrea, D.F.; Harwood, C.S. A chemosensory system that regulates biofilm formation through modulation of cyclic diguanylate levels. *Proc. Natl. Acad. Sci. USA* 2005, 102, 14422–14427.
115. Starkey, M.; Hickman, J.H.; Ma, L.; Zhang, N.; De Long, S.; Hinz, A.; Palacios, S.; Manoil, C.; Kirisits, M.J.; Starner, T.D.; et al. *Pseudomonas aeruginosa* rugose small-colony variants have adaptations that likely promote persistence in

the cystic fibrosis lung. *J. Bacteriol.* 2009, 191, 3492–3503.

116. Colvin, K.M.; Irie, Y.; Tart, C.S.; Urbano, R.; Whitney, J.C.; Ryder, C.; Howell, P.L.; Wozniak, D.J.; Parsek, M.R. The Pel and Psl polysaccharides provide *Pseudomonas aeruginosa* structural redundancy within the biofilm matrix. *Environ. Microbiol.* 2012, 14, 1913–1918.
117. Wang, S.; Parsek, M.R.; Wozniak, D.J.; Ma, L.Z. A spider web strategy of type IV pili-mediated migration to build a fibre-like Psl polysaccharide matrix in *Pseudomonas aeruginosa* biofilms. *Environ. Microbiol.* 2013, 15, 2238–2253.
118. Wang, S.; Yu, S.; Zhang, Z.; Wei, Q.; Yan, L.; Ai, G.; Liu, H.; Ma, L.Z. Coordination of swarming motility, biosurfactant synthesis, and biofilm matrix exopolysaccharide production in *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* 2014, 80, 6724–6732.
119. Periasamy, S.; Nair, H.A.S.; Lee, K.W.K.; Ong, J.; Goh, J.Q.J.; Kjelleberg, S.; Rice, S.A. *Pseudomonas aeruginosa* PAO1 exopolysaccharides are important for mixed species biofilm community development and stress tolerance. *Front. Microbiol.* 2015, 6, 851.
120. Ma, L.; Lu, H.; Sprinkle, A.; Parsek, M.R.; Wozniak, D.J. *Pseudomonas aeruginosa* Psl is a galactose- and mannose-rich exopolysaccharide. *J. Bacteriol.* 2007, 189, 8353–8356.
121. Flemming, H.C.; Wingender, J.; Szewzyk, U.; Steinberg, P.; Rice, S.A.; Kjelleberg, S. Biofilms: An emergent form of bacterial life. *Nat. Rev. Microbiol.* 2016, 14, 563–575.
122. Jiang, K.; Li, W.; Zhang, Q.; Yan, G.; Guo, K.; Zhang, S.; Liu, Y. GP73 N-glycosylation at Asn144 reduces hepatocellular carcinoma cell motility and invasiveness. *Oncotarget* 2016, 7, 23530–23541.
123. Fett, W.F.; Osman, S.F.; Dunn, M.F. Characterization of exopolysaccharides produced by plant-associated fluorescent *Pseudomonads*. *Appl. Environ. Microbiol.* 1989, 55, 579–583.
124. Singh, S.S.; Devi, S.K.; Ng, T.B. Banana lectin: A brief review. *Molecules* 2014, 19, 18817–18827.
125. Bloemberg, G.V.; Lugtenberg, B.J.J. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr. Opin. Plant Biol.* 2001, 4, 343–350.
126. Chin-A-Woeng, T.F.C.; Bloemberg, G.V.; Mulders, I.H.M.; Dekkers, L.C.; Lugtenberg, B.J.J. Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. *Mol. Plant Microbe Interact.* 2007, 13, 1340–1345.
127. Lugtenberg, B.; Kamilova, F. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* 2009, 63, 541–556.