



Dissecting the process of xylem colonization through biofilm formation in *Erwinia amylovora*

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Abstract

Erwinia amylovora is the causal agent of fire blight, an economically-important disease affecting apple and pear production worldwide. Initial contact and infection of the host by *E. amylovora* mainly occurs in flowers, or in young leaves at actively-growing shoot tips. Infection via shoot tips encompasses several distinct steps which include the utilization of a Type III secretion system (T3SS) to establish bacterial populations within the apoplast, infection of the parenchyma, invasion of the xylem, attachment to xylem vessels, biofilm formation, and the eventual colonization of the xylem which manifests outwardly as wilting symptoms in the plant. After *E. amylovora* gains entry into the xylem, initial attachment to the xylem vessels is mediated by type I fimbriae. Conversely, the small RNA (sRNA) chaperone Hfq and associated sRNA ArcZ negatively regulate attachment and promote biofilm maturation. Attachment and biofilm formation within the xylem are enhanced by the mechanical force emerging from the flow of xylem sap. The second messenger molecule cyclic-di-GMP (c-di-GMP) regulates the transition into the biofilm phase of the infection process of *E. amylovora*. C-di-GMP also regulates the production of critical exopolysaccharides amylovan and cellulose, that lend to the structural stability and growth of biofilms within the xylem vessels. In this review, we provide an in-depth evaluation of the process of biofilm formation occurring within the host, as a result of *E. amylovora* infection. We also provide a model encompassing the different physical and signaling factors involved in biofilm initiation and maturation in *E. amylovora*, and highlight what needs to be done in the future.

Keywords *Erwinia amylovora* · Fire blight · Biofilm · Attachment · C-di-GMP · Flow · sRNA · Amylovan · Cellulose

Introduction

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is a disease of major economic importance in most pome fruit growing regions of the world. Studies of host-pathogen interactions in the fire blight pathosystem have been critically beneficial to the identification and the deployment of resistance genes and anti-virulence genes into elite breeding lines (Pompili et al. 2020; Vogt et al. 2013; Wohner et al. 2018). Likewise, examinations of virulence factors important to *E. amylovora* pathogenesis have identified new potential targets for disease management interventions.

Biofilm formation within the xylem is an important virulence factor contributing to *E. amylovora* pathogenesis. Biofilms enable *E. amylovora* to accumulate large populations during shoot infection, and facilitate systemic spread of *E. amylovora* through the host, which confers both short and long-term survival and virulence advantages to *E. amylovora*. Clustered within biofilms, *E. amylovora* populations within the xylem vessels can rapidly and significantly expand in a short period of time, enabling widespread systemic spread, extending into rootstock infection (Koczan et al. 2009; Momol et al. 1998). Biofilm development is an orchestrated process that involves several distinct steps that have been individually documented. We provide compiled evidence from *E. amylovora* and other plant pathogenic bacteria demonstrating the process of entry into the xylem through the parenchyma, the environmental triggers within the xylem that contribute to biofilm initiation and development, as well as the regulatory signaling components that regulate biofilm formation. We also explore the stages of biofilm formation, including attachment, which is mediated by various appendages including fimbriae and type

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IV pili, the process of biofilm maturation, and the physical components such as the exopolysaccharides (EPSs) necessary for the structural integrity of biofilms.

Why study biofilm formation in *E. amylovora*?

Biofilms serve as a universal bacterial adaptive mechanism that allow for successful niche habitation. The inclusion of biofilm development as a component in the overall disease cycle of pathogenic bacteria has been shown to lend several advantages in terms of survival, antibiotic resistance, etc. (Hall and Mah 2017). Biofilm formation in plant xylem has been well studied in several plant pathogenic bacteria, including *E. amylovora*, *Pantoea stewartii*, *Ralstonia solanacearum*, and *Xylella fastidiosa* (Castiblanco and Sundin 2016; Lowe-Power et al. 2018a, b; Mina et al. 2019; Roper 2011). Studies of these pathogens have revealed a wealth of information uncovering the mechanistic aspects of biofilm formation, genetic regulation of biofilm formation, and potential targets for anti-biofilm strategies for disease control. Plant hosts have also evolved mechanisms to inhibit pathogen biofilms in xylem, and studying these processes may yield information that can be used in plant breeding. As an example, the *R. solanacearum* / tomato system has provided insights into how biofilm formation within the xylem can serve as a limiting factor with regard to varietal differences in host resistance (Caldwell et al. 2017). In *X. fastidiosa*, research has highlighted the importance of metallic ions such as copper, as essential limiting factors for biofilm formation within the xylem, and that the host can regulate the concentrations of metal ions in xylem to limit pathogen colonization (Cobine et al. 2013). These aspects of host resistance as well as the factors modulating biofilm formation within the xylem are largely not understood in *E. amylovora*, and, if elucidated further, will provide a general basis to develop disease control strategies, and to further understand host-pathogen interactions pertaining to evolutionarily adaptive outcomes.

Entry into the xylem during *E. amylovora* infection

Flowers are the primary route of entry into the host for *E. amylovora* (Miller and Schroth 1972). Insect vectors such as flies can initially transfer *E. amylovora* from oozing cankers onto flower stigmas. The stigma is a site of rapid population expansion; under optimal conditions, populations of *E. amylovora* can reach 10^6 to 10^7 cfu per flower (Johnson et al. 2006; Thomson and Gouk 2003). The availability of free water on flowers then enables bacterial migration to the nectarhodes (Bubán and Orosz-Kovács 2003; Johnson et al. 1993). In contrast to flower infection, apple shoots infection

can be incited by <math><100</math> cells (Crosse et al. 1972). The observation of ooze droplets containing $\sim 10^8$ to 10^9 cells as soon as shoot infection symptoms become visible (Slack et al. 2017) indicates a requirement for some mechanism of population expansion following infection of leaves at shoot tips. One possibility for population growth could be through the establishment of biofilms in leaf xylem elements.

In leaves, movement of *E. amylovora* cells occurs through the epidermis, into the parenchymal tissue surrounding the xylem, and finally into the xylem vessel elements (Goodman and White 1981; Suhayda and Goodman 1981a, b). Infection through wounds at shoot tips also potentially provides direct access to xylem tissue, in addition to the route channeled through the adjacent parenchymal tissue. Extensive cell proliferation occurs in the intracellular regions of the parenchyma prior to the entry of *E. amylovora* into the xylem vessels (Goodman and White 1981; Suhayda and Goodman 1981b). The nutrient/osmotic components of the xylem sap are hypothesized to be the signals that contribute to *E. amylovora* cell movement towards the xylem in a chemotactic sensory response model, and the fluidic pressure differential between the parenchyma and the xylem vessels is thought to mitigate bacterial channeling into the xylem vessels (Bogs et al. 1998). During the early stages of entry into the xylem, despite the colonization of the adjacent parenchyma tissue, visual necrotization of external foliar tissue may be relatively delayed (Suhayda and Goodman, 1981). EPS production (mainly amylovoran and levan) is observationally associated with proliferation and movement within the cortical parenchyma (Goodman and White 1981; Suhayda and Goodman 1981b). A detailed understanding of the pathway through which the movement of bacteria occurs via parenchymal tissue and into the xylem vessels is generally not well understood in *E. amylovora* and, in other phytopathogenic bacteria like *P. stewartii* (Roper 2011), *R. solanacearum* (Peeters et al. 2013) and *Xanthomonas oryzae* (Nino-Liu et al. 2006). However, direct access to the xylem, to some degree, is provided by insect vectors for *P. stewartii* (Roper 2011) and *X. fastidiosa* (Newman et al. 2003). In *R. solanacearum* (Peeters et al. 2013; Vasse et al. 1995) and *X. oryzae* (Tabei and Mukoo 1960), the primary route of entry into the host is through damaged root tissue and hydathodes, respectively. Some of these entry routes might not grant direct access to xylem vessels and thus require movement through the parenchyma.

Factors contributing to attachment and biofilm formation in the xylem

Microfluidic flow Attachment to, and biofilm development within xylem vessels can be influenced by several chemical and mechanical factors within the xylem. The flow of xylem

sap mediated by transpiration generates shear force within the xylem vessels (Karam 2005). The level of this force is dependent on the flow rate and viscosity of the xylem sap, which can vary based on several factors including the developmental stage of the plant, the location of the xylem vessels within different plant organs, environmental factors like humidity and temperature, as well as the circadian rhythm of the plant (Karam 2005). The flow of xylem sap is a common regulator of biofilm formation in several xylem-dwelling bacterial phytopathogens including *R. solanacearum* (Lowe-Power et al. 2018a; b) and *X. fastidiosa* (Meng et al. 2005). Fluidic flow positively regulates biofilm formation in *E. amylovora* in vitro, relative to conditions where flow is absent (Kharadi and Sundin 2019; Koczan et al. 2009). However, the effect of varying levels of flow on biofilm formation by *E. amylovora* in a plant model system in real time has not been studied yet.

Organic metabolite composition of the xylem sap The xylem sap is a complex solution comprised of dissolved organic and inorganic compounds (Buhtz et al. 2004). The organic compounds include sugars, amino acids and organic acids. These compounds can play a role in sustaining bacterial growth within xylem vessels. *E. amylovora* requires a wide range of metabolites including purines, pyrimidines and sugars like sorbitol for growth and virulence in planta (Klee et al. 2019). However, a direct relationship between specific metabolites and their effect on biofilm formation either in vitro or within the xylem has not yet been established for *E. amylovora*. Of interest, *R. solanacearum* has been shown to modify the organic composition of the xylem by producing, and thereby elevating the levels of a polyamine, putrescine (a carbon and nitrogen source), post infection within the xylem sap (Lowe-Power et al. 2018a; b). Putrescine levels within the xylem sap had a proportional effect on bacterial growth, biofilm formation and wilting symptoms within infected plants (Lowe-Power et al. 2018a, b).

Xylem ionome composition The inorganic composition of the xylem sap, including minerals and trace elements, can be collectively referred to as the xylem ionome (Salt et al. 2008). The xylem ionome can vary significantly in response to various physiological factors affecting the plant, and also as a result of bacterial colonization (Salt et al. 2008). In the shoot infection model of fire blight, iron acquisition using siderophores such as desferrioxamine is critical for pathogenicity in *E. amylovora* (Dellagi et al. 1998). Across different apple cultivars (*Malus x domestica*) with varying susceptibility to fire blight, a comparative analysis of cytosolic calcium accumulation (Ca^{2+}) indicated that resistant cultivars displayed increased Ca^{2+} accumulation over time, as compared to the more susceptible cultivars, in response to mechanical damage or *E. amylovora* infection (Kanchiswamy et al. 2013). A comprehensive study of the effect of the individual

components and the collective xylem ionome is lacking in *E. amylovora*. In response to a *X. fastidiosa* infection, the leaf ionome was found to be significantly modified, with an enhancement in the level of calcium and phosphorous (De La Fuente et al. 2013). Zinc homeostasis and zinc detoxification were also found to be critical for *X. fastidiosa* growth and EPS production in xylem sap, and under microfluidic flow-based conditions, an increased level of zinc in the medium was correlated with the production of denser biofilms in vitro (Navarrete and De La Fuente 2015).

Attachment and biofilm development within the xylem vessels

Bacterial attachment appendages In order to initiate biofilm formation, the first step entails the movement of free-swimming planktonic bacterial cells towards the target attachment surface and making initial contact with the surface. In other organisms including *Pseudomonas aeruginosa* (Barken et al. 2008; O'Toole and Kolter 1998), *Escherichia coli* (Prigent-Combaret et al. 2000; Wright et al. 2005) and *Pectobacterium carotovorum* (Hossain and Tsuyumu 2006), the flagellum initiates this contact with the surface and is necessary for biofilm development. In *E. amylovora*, absence of the flagellar regulators FlhD and FlhC, along with flagellar structural component proteins FlgA-N resulted in a negative effect on surface attachment in vitro (Koczan et al. 2011). However, other flagellar filament proteins FliE-Q showed inconclusive results on their involvement in attachment (Koczan et al. 2011). Further, during the initiation of biofilm formation, the primary attachment to a surface can either be reversible or irreversible. Attachment as a result of flagellar contact with a surface is usually reversible, and thus anchoring mediated by other appendages like type IV pili and fimbriae is required to strengthen this surface interaction (Monds and O'Toole 2009). For *E. amylovora*, in-vitro surface attachment for biofilm initiation was experimentally estimated to occur within the first 2 h of contact with a surface. In this phase, type I fimbriae were the critical extracellular appendages responsible for surface attachment, under static and flow-based conditions (Koczan et al. 2011). The interdependence of fimbrial attachment on initial surface contact by flagellar activity has not been examined in *E. amylovora*.

The next phase of the process of biofilm initiation is that of irreversible attachment to a surface. Type IV pili mediate this in *E. amylovora* following initial attachment to a surface, potentially through the combined effect of flagellar and fimbrial activity (Koczan et al. 2011). Disrupting the type IV pilus transport protein HofC in *E. amylovora* resulted in significantly reduced irreversible surface attachment in-vitro. The initial reversible attachment was not affected by the deletion of *hofC*; however, further biofilm development was significantly

reduced as a result (Koczan et al. 2011). Curli fimbriae were also found to positively regulate biofilm maturation in *E. amylovora* (Koczan et al. 2011). Determining the sequential codependence of the flagellar, type I curli fimbrial, and type IV pili mediated surface contact and attachment will be critical to fully understand the initial progression of biofilm formation by *E. amylovora* in the xylem vessels.

Exopolysaccharides Amylovoran, levan, and cellulose are the three major exopolysaccharides produced by *E. amylovora*, and all of these exopolysaccharides contribute to the biofilm matrix (Bernhard et al. 1993; Castiblanco and Sundin 2018; Gross et al. 1992). Amylovoran is a heteropolymer with glucose, galactose, and pyruvate as the compositional substrates (Nimtz et al. 1996). Amylovoran biosynthesis and secretion are functionally controlled by the products of the 12-gene *ams* operon (Bernhard et al. 1993). *E. amylovora* mutants lacking amylovoran production are incapable of forming biofilms in vitro (Koczan et al. 2009). *E. amylovora ams* mutants are also nonpathogenic, although non-*ams* mutants reduced in biofilm formation retain pathogenicity but are reduced in virulence (Koczan et al. 2009, 2011).

Levan, a heteropolymer comprised of fructose subunits, is generated as a result of the enzymatic breakdown of sucrose by the levansucrase enzyme (Gross et al. 1992). Levan partially contributes to virulence in *E. amylovora* (Gross et al. 1992). Surface attachment and biofilm formation under flow, are also dependent on levan, although, amylovoran regulates these factors strongly (Koczan et al. 2009). Biofilm development within the xylem vessels is also dependent on levan production and, the inability to synthesize levan limits *E. amylovora* colonization to the parenchymal tissue (Koczan et al. 2009).

Cellulose, a glucose-based EPS, was only recently linked to the biofilm matrix in *E. amylovora* (Castiblanco and Sundin 2018). Cellulose manifests as fibrillar material in the extracellular biofilm matrix. The inability to generate cellulose has an impact on the architecture of the biofilm matrix in vitro and in planta, and the colonization of the xylem vessels is not fully eliminated (Castiblanco and Sundin 2018). The production of multiple EPSs that contribute to virulence, biofilm formation, and host colonization is also characteristic of plant pathogenic pseudomonads and xanthomonads (Rudolph et al. 1994).

Regulatory factors influencing biofilm formation in *E. amylovora*

Cyclic di-GMP The ubiquitous bacterial second messenger molecule bis-(3',5')-cyclic diguanosine monophosphate (c-di-GMP) regulates the transition to attachment and biofilm

formation from a comparatively motile lifestyle. Diguanylate cyclase enzymes (Edcs) and phosphodiesterase enzymes (Pdes) enzymatically synthesize and hydrolyze c-di-GMP, respectively (Jenal et al. 2017). In *E. amylovora*, elevated intracellular levels of c-di-GMP positively regulate amylovoran and cellulose production, while negatively regulating flagellar motility (Castiblanco and Sundin 2018; Edmunds et al. 2013; Kharadi et al. 2019; Zhao and Sundin 2017). Studies to understand the action of varying intracellular levels of c-di-GMP in *E. amylovora* have been conducted through the targeted interruption of single or multiple *edc* and *pde* genes. The reduction in c-di-GMP levels as a result of the combined elimination of *edcC* and *edcE* resulted in a significant reduction in biofilm formation in vitro. Correspondingly, *edcC* and *edcE* also contribute to amylovoran and cellulose production, which impacted biofilm formation (Castiblanco and Sundin 2018; Edmunds et al. 2013). In addition, deleting any two of the three Pde enzyme encoding genes (*pdeA*, *pdeB*, or *pdeC*) resulted in a two-fold or greater increase in intracellular c-di-GMP levels, that led to increased amylovoran production and biofilm formation under static or flow-based conditions in vitro (Kharadi et al. 2019; Kharadi and Sundin 2019). In the *E. amylovora* $\Delta pdeABC$ triple mutant, intracellular levels of c-di-GMP were elevated to an almost ten-fold higher concentration compared to the WT strain (Kharadi et al. 2019). This resulted in an autoaggregation phenotype and impaired cell separation post cell division in liquid growing conditions (Kharadi and Sundin 2019). Amylovoran and cellulose were also found to contribute to the formation of *E. amylovora* cell aggregates. Autoaggregation severely impaired biofilm formation under relatively static conditions, presumably due to reduced interaction of the aggregated cells with a surface. Under flow, however, biofilm formation recovered to some degree, despite the presence of autoaggregation (Kharadi and Sundin 2019). Collectively, these data indicate the importance of accurate regulation of intracellular c-di-GMP levels to control attachment and biofilm formation in *E. amylovora*.

The spatiotemporal environmental stimuli within the host that regulate c-di-GMP production to trigger attachment and biofilm formation are yet to be fully understood in the context of an in-planta *E. amylovora* infection. Also, the attachment appendages that are under c-di-GMP regulation are unknown in *E. amylovora*. Other phytopathogenic bacteria such as *P. syringae* (Aragón et al. 2015; Pfeilmeier et al. 2016) and *X. campestris* (Lu et al. 2012; Ryan et al. 2007) have regulatory models similar to *E. amylovora*, with c-di-GMP positively regulating EPS production and biofilm formation. In contrast, in *X. fastidiosa*, c-di-GMP is a negative regulator of these two processes (Chatterjee et al. 2010; De Souza et al. 2013).

Hfq and small noncoding regulatory RNAs The Hfq-dependent subset of small non-coding RNAs (sRNAs) in *E. amylovora* were found to be involved in various stages of biofilm formation. Firstly, the deletion of the chaperone Hfq (*hfq* gene) resulted in a hyper-attachment phenotype under in-vitro static conditions (Zeng et al. 2013; Zeng and Sundin 2014). This, combined with the very similar phenotype observed when the sRNA ArcZ was eliminated, suggests that ArcZ negatively regulates attachment during biofilm initiation, and that the regulatory effect necessitates chaperoning by Hfq. The elevated attachment in the Δhfq and $\Delta arcZ$ mutant strains did not translate to increased overall biofilm formation, and instead ceased at the attachment stage of the process. This suggests that Hfq/ArcZ might regulate biofilm development, potentially through their positive regulatory effect on the production of amylovoran, which comprises of the bulk of the biofilm matrix (Zeng and Sundin 2014). In *X. campestris*, Hfq was shown to positively regulate EPS production and virulence (Lai et al. 2018). Likewise, in *P. ananatis* (Shin et al. 2019), Hfq also positively regulates biofilm formation. The regulatory pathway by which Hfq/ArcZ impact attachment as well as the attachment appendage that is impacted in *E. amylovora* during biofilm initiation and development is not fully understood.

Quorum sensing In several other plant pathogens including *R. solanacearum* (Flavier et al. 1997; Kai et al. 2015), *X. fastidiosa* (Ionescu et al. 2013) and *P. carotovorum* (Andersson et al. 2000; Burr et al. 2006), quorum sensing is a key factor driving regulatory shifts that enable the pathogen to promote biofilm formation. In *E. amylovora*, however, the LuxS homolog (involved in autoinducer (AI) synthesis in quorum sensing bacteria) has been reported to have conflicting functions depending on the background strain(s) used for experimentation (Gao et al. 2009; Rezzonico and Duffy 2007). Collectively, these reports suggest that LuxS synthesizes an AI-2 class of autoinducers that has some effect on EPS production and virulence (Gao et al. 2009). However, a bioinformatic search of the genome of several *E. amylovora* strains did not indicate the presence of any proteins that had an AI-2 receptor that might indicate responsiveness towards the autoinducer (Rezzonico and Duffy 2007). The major effect of LuxS seems to be towards regulating metabolism under sulfur-limiting conditions, not quorum sensing (Rezzonico and Duffy 2007). Thus, quorum sensing has not been implicated in regulating biofilm formation in *E. amylovora*.

Detachment from biofilms

In the *E. amylovora* system, the process of detachment of cells from the biofilm matrix has not been explored yet. A detailed

investigation of the process must include factors like the regulator(s) involved mediating the switch from an attached to a detached lifestyle, the physical process of separation from the matrix, and, the spatiotemporal location of the detachers and their subsequent movements towards re-establishing new biofilms/ other trajectories. In *R. solanacearum*, PhcA is a virulence regulator that responds to quorum sensing and mediates the switching between aggregation and dispersal of cells from a preformed biofilm (Khokhani et al. 2017). This regulation represents niche adaption to promote optimal resource utilization and survival. In *X. campestris*, the *rpf* gene cluster, regulated by diffusible signal factor molecules, mitigates dispersal of cells from a biofilm aggregate through the enzymatic action of an endo- β -1,4-mannanase (Dow et al. 2003). In *Pseudomonas fluorescens*, the acyl homoserine lactone (AHL) class of autoinducers (studied by the exogenous addition of AHL) regulate dispersal of cells from a biofilm through the production of an exopolysaccharide lyase enzyme (Allison et al. 1998). Overall, these regulatory patterns could provide some basis for the exploration of homologous factors pertaining to dispersal from biofilms within the *E. amylovora* system.

Overall model for biofilm development in *E. amylovora*

A current model for biofilm development in xylem by *E. amylovora* is shown in Fig. 1. Entry of *E. amylovora* cells into plant tissue can occur via a floral or an epidermal route (Bogs et al. 1998; Goodman and White 1981; Suhayda and Goodman 1981a, b). In certain cases, mechanical injury (to epidermal tissue) might lead to direct xylem access, however, *E. amylovora* cells often move through apoplastic tissue, invade and colonize the parenchyma surrounding xylem vessels, and finally enter xylem vessels (Bogs et al. 1998; Goodman and White 1981; Suhayda and Goodman 1981a and b). The organic and inorganic composition of the xylem, along with the shear stress present within the xylem vessels due to flow resulting from transpiration, together act as the sensory signals that trigger attachment to the walls of the xylem vessels (Dellagi et al. 1998; Kanchiswamy et al. 2013; Kharadi and Sundin 2019; Klee et al. 2019). While some flagellar components are thought to initiate surface contact, type I fimbriae are the primary bacterial appendages that mediate initial surface attachment. Additionally, type IV pili and curli fimbriae were found to contribute to biofilm development and maturation (Koczan et al. 2011). Cyclic di-GMP levels are elevated intracellularly in response to the environmental signals within the xylem and regulate biofilm formation. Cyclic di-GMP positively regulates the production of amylovoran and cellulose, but not levan, although levan is also a critical EPS contributing to biofilm formation (Castiblanco and Sundin 2018; Edmunds et al. 2013; Gross

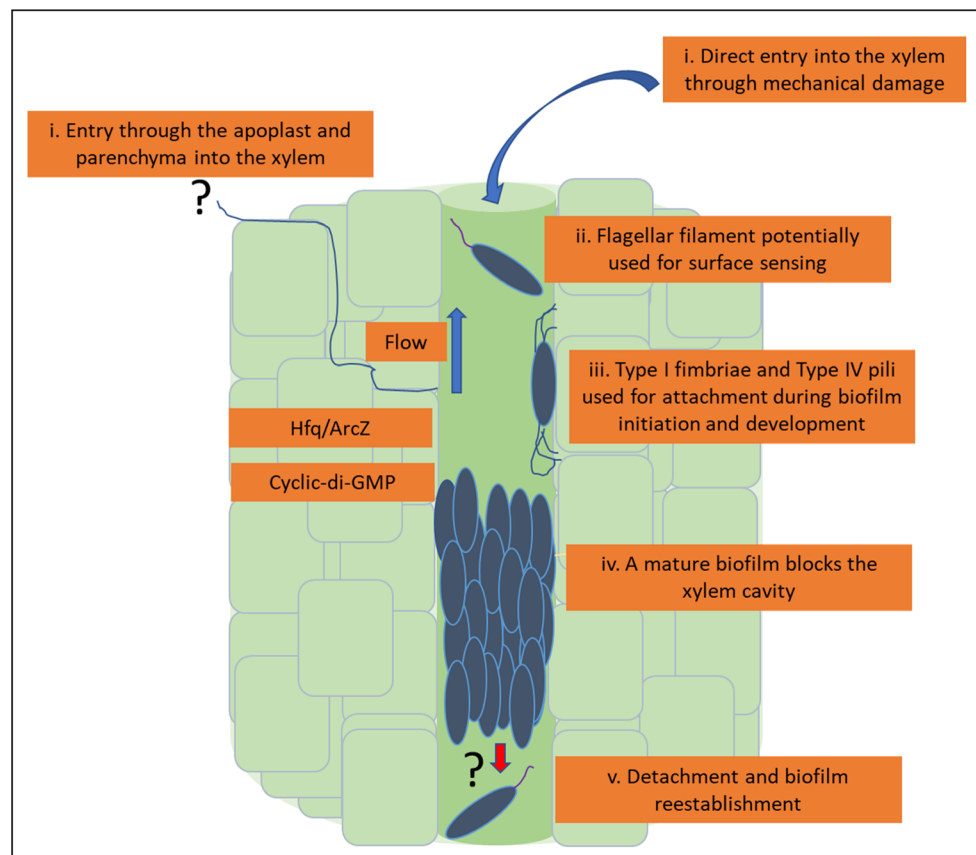


Fig. 1 A pictorial flow depicting various stages leading up to biofilm formation and its development and progression for *E. amylovora* within the host tissue. **i.** Entry through epidermal tissue due to mechanical tissue damage may grant *E. amylovora* cells direct entry into the xylem. **ii.** Movement through the apoplast and the parenchymal tissue surrounding the xylem is required if *E. amylovora* cells enter through floral or epidermal tissue and can't directly enter the xylem. The mechanics of this process are not fully understood. **iii.** Some flagellar components could be involved in surface sensing after the initial entry of *E. amylovora* cells into the xylem. **iv.** Initial attachment to the walls of the xylem vessel is primarily mediated through type

I fimbriae. Type IV pili are also required for biofilm development. The chronology of their implementation during the process of biofilm formation has not been studied in real time. **v.** As the biofilm within the xylem matures, the flow of xylem sap is physically impeded. The mechanical force originating flow within the xylem vessel is an important sensory cue to promote biofilm development. C-di-GMP and sRNA regulation in the form of Hfq/ArcZ are significant in biofilm development due to their impact on EPS production. **vi.** Detachment from a mature biofilm and the downstream actions that follow pertaining to biofilm spread within the xylem are not well-understood

et al. 1992; Kharadi et al. 2019; Kharadi and Sundin 2019). The sRNA ArcZ along with the chaperone protein Hfq negatively regulate attachment, and positively regulate amylovoran production, with a downstream effect on biofilm development (Zeng et al. 2013; Zeng and Sundin 2014).

Conclusions and perspectives

Biofilm formation is a key determinant of successful host colonization and is an important step in the fire blight disease cycle, wherein *E. amylovora* can expand its population within the host, leading to enhanced disease progression. The process of biofilm formation requires the successful serial execution of several regulatory steps within the broad categories of

Table 1 A suggested list of future research areas to focus on to gain further insights into the process of biofilm development and host colonization in *E. amylovora* system

The physiochemical process of the movement of <i>E. amylovora</i> through plant tissues and entry into the xylem
The regulatory factor(s) within <i>E. amylovora</i> involved in mediating the switch to biofilm formation upon entry into the xylem
Documenting the process of attachment via an external appendage(s) within the xylem in real time, while accounting for the environmental triggers within the xylem that influence this occurrence
Understanding the mechano-physical process and the regulatory pathways underlying detachment of cells from a biofilm within the xylem
The spatiotemporal dynamics of biofilm development and the progression of infection through the xylem vessels
A transcriptomic, proteomic and metabolomic profiling of the host and the pathogen simultaneously in a biofilm within the xylem to gain a well-rounded understanding of host-pathogen interactions in this context

preliminary xylem invasion, attachment to the xylem vessels, biofilm development and detachment from the biofilm for reinfection. While our review highlights the specific details of this process that have been documented for *E. amylovora*, further research will be required to fully understand the other stages of this process. In Table 1, we present some regulatory categories that need further study.

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