Plant cell wall polysaccharides induce colony expansion of soil-derived *Flavobacterium* spp.

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Running Head: Pectin induce Flavobacteria colony expansion by TonB proteins

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Abstract

Flavobacterium is a genus of gram-negative bacteria, belonging to the *Bacteriodetes* phylum, characterized by a unique gliding motility. They are ubiquitous and often abundant in root microbiomes of various plants, but the factors contributing to this high abundance are currently unknown. In this study, we evaluate the effect of various plant-associated poly- and mono-saccharides on growth and colony expansion of two *Flavobacterium* strains (*F. Johnsoniae*, *Flavobacterium* sp. F52). Both strains were able to grow on pectin and other polysaccharides such as cellulose as a single carbon source. However, only pectin, a polysaccharide that is profuse in plant cell walls, enhanced colony expansion on solid surfaces even under high nutrient availability, suggesting a link between carbohydrate metabolism and gliding. Expansion on pectin was dose- and substrate-dependent, as it did not occur when bacteria were grown on the pectin monomers galacturonic acid and rhamnose. Using time-lapse microscopy, we demonstrated a bi-phasic expansion of *F. johnsoniae* on pectin: an initial phase of rapid expansion, followed by biomass production within the colonized area. Proteomics and gene expression analyses revealed significant induction of several carbohydrate metabolism related proteins when *F. johnsoniae* was grown on pectin, including selected operons of SusC/D, TonB-dependent glycan transport genes. Our results suggest a yet unknown linkage between specific glycan associated operons and flavobacterial motility. This may be associated with their capacity to rapidly glide along the root and metabolize plant cell wall carbohydrates, two characteristics that are crucial to rhizosphere competence.

Importance

The genus *Flavobacterium* is highly abundant and enriched (relative to surrounding bulk soil) in plant root microbiomes, where they may play a role in plant health and ecosystem functioning. However, little is known about genetic and physiological characteristics that enable flavobacteria to colonize and proliferate in this highly competitive environment. In this study, we found that plant cell wall-polysaccharides and specifically pectin stimulate flavobacteria colony development in a bi-phasic manner, initially characterized by rapid expansion followed by increased biomass production. This appears to be linked to pectin-facilitated induction of specific TonB-associated proteins evidentially involved in the detection and uptake of plant sugars. These findings suggest that the capacity to sense, expand on and metabolize pectin and other plant cell wall polysaccharides play a fundamental role in promoting rhizosphere competence in flavobacteria. This work sheds light on specific mechanisms that facilitate plant-microbe interactions, which are fundamental for promoting plant health and for understanding the microbial ecology of root ecosystems.

Keywords: Flavobacterium, Roots, Rhizosphere, Pectin, TonB

Introduction

The complex interactions between plant-associated microorganisms and their hosts (collectively referred to as the "plant holobiont") are crucial for plant health and growth ((1–3). Plants modulate the narrow region of soil that is influenced by root secretions, the rhizosphere, by exuding various small molecular weight compounds (rhizodeposites) such as sugars, amino acids and organic acids, by rhizodepositing root cap border cells, and by releasing various mono- and poly-saccharides in their mucilage (3–8). Organic compounds released from the roots transform the rhizosphere into a nutrient rich environment relative to the surrounding bulk soil, which facilitates colonization by soil microorganisms. Rapid growth, coupled to competition for root resources, results in less diverse rhizosphere and rhizoplane microbiomes relative to surrounding bulk soil, a phenomenon known as the "rhizosphere effect" (4, 9, 10). Rhizosphere and root colonizing bacteria have the capacity to outcompete other soil bacteria and thereby proliferate and thrive in the root ecosystem through a combination of specific traits collectively coined "rhizosphere competence", which include: motility, resistance to stress, ability to utilize plant-derived organic compounds, chemotaxis, and the production of secondary metabolites (5). Some earlier studies demonstrated that rhizosphere bacteria are attracted to the roots by plant-exuded organic acids such as malic, citric or fumaric acid and various amino acids (11–15). Many of these sensed chemo-attractants can also be consumed by the bacteria (16). Although rhizosphere recruitment and colonization mechanisms of certain plant-growth-promoting rhizobacteria (PGPR) have been identified and characterized, those responsible for recruitment of the vast majority of rhizosphere and rhizoplane bacteria is currently an enigma (17–19).

Flavobacterium is a Gram-negative genus of bacteria from the phylum *Bacteroidetes* known to degrade complex organic compounds, which is abundant in freshwater, marine and soil environments (20, 21)). It is highly abundant in the rhizoplane, rhizosphere and phylosphere of a wide array of plants, in contrast to considerably lower abundance in bulk soil (21–28), suggesting that members of this genus have specifically adapted to root ecosystems. Several root- and soil-derived flavobacteria were found to antagonize various plant pathogens in different crops (29–33), and recently it was discovered that entophytic *Flavobacterium* strains play a fundamental role in antagonizing phytopathogenic fungi, through the production chitinolytic enzymes and specific secondary metabolites (34). Furthermore, they were recently identified as potential driver taxa behind pathogen suppression in analysis of bacterial network in a plant root microbiomes (35). In addition, selected members of this genus are linked to increased plant biomass, and have therefore been defined as plant growth promoting rhizobacteria (PGPR) of various crops (23, 29, 30, 36–38).

Soil flavobacteria have specialized ability to decompose complex plant derived polysaccharides, such as pectin and cellulose and the ability to secret various carbolytic enzymes via the *Bacteriodetes*-specific type IX secretion system (T9SS) (21, 39–43). Like other *Bacteroidetes*, they contain a myriad of genes that encode Polysaccharide Utilization Loci (PULs) that are activated specifically to facilitate glycan capture and intake (44). These PULs include outer membrane protein transducers involved in polysaccharide utilization, which are part of the TonB family, generally referred to as Starch Utilization System (SUS) proteins. Interestingly, comparative genomics revealed that genomes of soil and root-associated flavobacterial strains are significantly enriched with genes associated with plant polysaccharide degradation relative to

aquatic strains from this genus, indicating that physiology of this genus is strongly influence by its ecology (21).

Most terrestrial *Flavobacterium* strains possess a unique gliding mechanism that rapidly propels them over solid and semi-solid surfaces. The proteins that comprise this gliding system are molecularly intertwined with at least fifteen proteins that make up the T9SS, seven of whom are responsible for the secretion of SprB and RemA adhesins which are expressed on the cell surface and involved in gliding (45, 46). We previously demonstrated that this T9SS-gliding complex is crucial for root colonization by flavobacteria, and this colonization was positively linked to the induction of plant resistance to foliar pathogens (31).

Collectively, the above data strongly suggest that terrestrial flavobacterial strains have evolved means that enable them to interact with plant roots, and that these interactions are beneficial to plant health. Nonetheless, the specific mechanisms behind this phenomenon are currently unclear.

In this study, we assessed the impact of various plant-derived sugars on the motility and growth dynamics of flavobacteria by coupling conventional petri dish assays and live-imaging fluorescent microscopy with proteomic and gene expression analyses. This study demonstrates that pectin, a plant cell wall-associated polysaccharide, facilitates bi-phasic proliferation over solid surfaces through induction of specific TonB-associated glycan uptake operons. *In-planta* extrapolation of these results, suggests that this link between pectin, motility and carbohydrate metabolism may be fundamental to rhizosphere competence in flavobacteria.

Results

Growth of *Flavobacterium* strains on various carbon sources

We previously demonstrated that genomes of terrestrial flavobacteria are enriched with genes involved in plant carbohydrate metabolism (21). To better understand the interaction of flavobacteria with sugars, growth was evaluated on minimal and rich media amended with the selected mono- and polysaccharides described. *F. johnsoniae* was motile and grew on chitin, pectin and cellulose but not on arabinose, as a single carbon source, (**FigS1**). Subsequently, we evaluated growth of flavobacteria on rich media (PY2 agar) coated with selected sugars (**Fig1A**). Colony expansion of *F. johnsoniae* on pectin was found to be significantly greater than the other analyzed sugars, and was five times higher than DDW ($p>0.05$, Tukey-Kramer HSD test) (**Fig1B**).

Plant cell wall polysaccharides expedite flavobacterial colony expansion

Wild type (WT) and gliding/typeIX secretion system mutants (ΔgldJ) of *F. johnsoniae* and the pepper root isolate *Flavobacterium* sp. F52 were inoculated in the center of PY2 agar media amended with DDW or pectin (**Fig1C**). After 48 hours of incubation, significant colony expansion of wild type bacteria was observed in both *Flavobacterium* strains when grown on pectin, while no similar growth was found on DDW. In contrast, gliding mutants (ΔgldJ) of both flavobacterial strains did not proliferate differentially on any of the plates (**Fig1C,D**), indicating that the gliding apparatus is a prerequisite for pectin-induced colony expansion.

Dose-dependent pectin facilitated colony expansion

To determine whether expansion on pectin is dose dependent, *F. johnsoniae* and *Flavobacterium* sp.F52 strains were inoculated at the center of PY2 agar media plates streaked with pectin at final concentrations of 0.5, 1, 2 and 4% percent. For all the examined pectin concentrations, colonies radiated along the pectin streaks, with 2% and

4% pectin concentrations inducing significantly more expansion than the lower concentrations ($p>0.05$,Tukey-Kramer HSD test) (**FigS2**).

Since galactronic-acid and rhamnose are the two major components of pectins, we examined the colony expansion of *F. johnsoniae* on rich media plates coated with DDW, 2% titrated galactronic-acid, 2% rhamnose, and a combination of galactronic acid and rhamnose. *F. johnsoniae* expansion on galactronic-acid, rhamnose or both of them together did not result in significant colony expansion ($p>0.05$,Tukey-Kramer HSD test), in contrast to bacteria grown on pectin (**Fig2A, B**).

Temporal dynamics of pectin induced *F. johnsoniae* colony expansion

The expansion of GFP labeled *F. johnsoniae* on PY2 agar coated with glucose, cellulose, glucomannan or pectin, was visualized at a higher resolution using time-lapse microscopy. Colony morphology after 32 hours, on each tested substance is presented in **Fig3A**.

Growth dynamics were clearly affected by the sugar type. Expansion on pectin was characterized by a relatively long lag phase. However following this stage, colonies rapidly expanded and after 18 hours, the fluorescent signal of colonies grown on pectin surpassed both the control (DDW) and the other amended compounds (according to Tukey-Kramer HSD test). Growth on pectin facilitated multiple ring-like structures that resemble previously described “raft” structures (47) (**Fig3B, Supplementary movie 1**). Conversely, expansion of colonies grown on glucose or DDW did not display this bi-phasic expansion (**Supplementary movie 1**). Bacteria growth on glucose expanded the least from 8 hours and throughout the experiment, resulting in compact, small colonies with less ringed structures, suggesting that glucose has an inhibitory effect on *F. johnsoniae* motility and possibly growth (**Fig3C**). The most significant expansion

observed between 6 to 20 hours was on cellulose, but the colonies grown on glucomannan and pectin proliferated at later times, and the colonies grown on pectin reached the greatest intensity at 44 hours (**Fig3C**), thereby surpassing the colonies grown on other sugar sources ($p>0.05$,Tukey-Kramer HSD test).

Colonies grown on pectin were characterized by a bi-phasic growth with initial expansion, followed by more significant growth phase (**Supplementary movie 1**) with an initial peak in fluorescence at 20 hours and a second peak at 36 hours (**Fig3D**). After 20 hours of growth, total fluorescence was highest in cells grown on pectin, glucomannan and cellulose, and lowest on glucose ($p>0.05$,Tukey-Kramer HSD test).

Next, we estimated the velocity of colony expansion on the selected sugars by measuring the time it took the colonies to cross three radials (3mm, 6mm and 9mm), and subsequently calculating the mean velocity from circle to circle (**Fig3E**). Initially, in the first 1.5mm radius the estimated velocity was similar on all substances. Between 1.5-3mm colony expansion on pectin and glucomannan increased. Colony expansion velocity slowed slightly in the outer circle with pectin, glucomannan still exhibiting the fastest expansion rate of all the tested sugars (**Fig3F**). Thus, *F. johnsoniae* expanded faster on pectin then on any of other tested sugars (**Fig3G**). Collectively, we conclude that growth of *F. johnsoniae* on pectin is bi-phasic. Following an initial lag phase, there is a rapid expansion phase where the colonies proliferate, followed by a slower growth phase where cells appear to proliferate less and replicate more and gain biomass. This replication step is significantly more intense with pectin than with any other carbon source.

Specific TonB/Sus transducers are expressed in response to growth on pectin

In order to gain deeper insight into the molecular mechanisms associated with flavobacteria colony expansion on pectin, we conducted a proteomic assay in which we examined differential intracellular protein expression after 44 hr growth between *F. johnsoniae* growing on rich media coated with pectin or DDW. Eighty-three proteins were found to be upregulated and forty-three were down regulated in the presence of pectin (**TableS1**). Genomic annotation with various tools revealed a high proportion of unassigned proteins, while a large fraction of the proteins (17%, 22% and 37% of KEGG, SEED and EggNog annotations, respectively) were associated with carbohydrate metabolism (**FigS3A-C**). Of the 25 most markedly pectin-induced proteins identified, 13 were involved in polysaccharide uptake, processing and metabolism, including four Sus C/D related proteins (**Fig4A**). Other pectin-induced proteins included a novel transcriptional regulator (upregulated 12-fold) and a protein associated with Auxin-regulation (upregulated 26-fold) (**Table S1**). Interestingly, none of the differentially expressed proteins were gliding related (**Table S2**).

Of the 44 previously-described SusC and 42 SusD homologues identified in the *F. johnsoniae* genome (48), 27 SusC and 15 SusD proteins were detected in the proteomic analysis, in addition to 610 flanking genes surrounding these Sus proteins that constitute PUL clusters seemingly associated with glycan metabolism. Of these, three SusC and six SusD proteins along with 31 associated PUL proteins (forming 4 gene clusters **Table S3**) were significantly induced in response to growth on pectin (**Table S4**).

In order to validate the proteomic results, *F. johnsoniae* cells were grown on pectin and DDW, and the expression of 8 genes (**Table S5**) was evaluated by quantitative real time PCR. Pectin did not facilitate expression of RemA involved in gliding adhesion (not evaluated in the proteomics since it's an extra-membranal protein), indicating that this protein is not associated with the induced colony expansion of flavobacteria on pectin

at the tested time point. Expression of genes encoding for the novel transcriptional regulator (Trans. Regul), Pectate lyase (Pec lyase), putative Auxin-regulated annotated protein (Auxin) and TonB 2144 was also not augmented by pectin despite the fact that they were significantly induced in the proteomic analysis. Among the examined TonB/SusC related genes, TonB 260 and Sus73 were significantly upregulated (60-100 fold, $p<0.05$ Tukey-Kramer HSD test) while TonB 445 was substantially upregulated (10-20 fold) but not statistically significant (**Fig4B**). TonB260 and Sus73 are part of an 18-gene cluster, of which all of the proteins were upregulated in the presence of pectin in the proteomic analysis (**cluster 1-Table S3**). Using two different prediction tools, the TonB260 and Sus73 encoding genes were mapped to the same operon together with a gene encoding for the hydrolytic enzyme polygalacturonase that cleaves the α (1-4) bonds between adjacent galacturonic acids within the homogalacturonic acid backbone of pectin (**Table S6**).

Discussion

Roots modulate their microbiome by secretion of exudates, mucilage and other components that can attract or deter bacteria (8, 47, 49). Specific root exudates were found to serve as chemoattractants for microbes colonize the roots (50). However, this seems to be the tip of the iceberg, and little is known about the plant-associated factors that recruit bacteria from the ecologically complex rhizosphere to the surface and endosphere of roots.

Bacterial plant root colonization is a multi-stage process that involves recognition, movement towards plant/seed attractants and attachment (15). Once attached, persistence and propagation on plant roots is dependent on metabolic fitness (51, 52)**,** resistance to plant protective mechanisms and inter-microbial interactions. The

abundance of bacteria in rhizosphere soil is about 10^2 higher than in the rhizoplane and root endosphere (53, 54). This exemplifies the extreme selective pressure of this highly competitive environment and the fitness requirements of the root-colonizing microbiome**.**

Members of the genus *Flavobacterium* are highly abundant and substantially enriched (relative to the rhizosphere soil) on, and within roots of several plant species (25, 32, 33, 55, 56), and certain strains have been found to augment plant health and suppress pathogens in a variety of plants (29, 32, 33, 35). In this study, we explored the effect of plant-derived sugars on surface expansion of flavobacteria, in attempt to elucidate mechanisms associated with plant root-flavobacterial interactions. It was previously shown that *F. johnsoniae* can effectively grow on polysaccharides such as pectin and glucomannan as single carbon sources (48). Collectively, results from this study demonstrate that flavobacteria not only metabolize complex plant cell wall-derived glycans such as pectin, glucomanan and cellulose, but that these sugars (particularly pectin) serve as cues for rapid spread of flavobacteria over solid surfaces. We postulate that this plant cell wall glycan recognition-uptake mechanism is critical for colonization and proliferation of flavobacteria on plant roots.

Interestingly, our results demonstrated that even when carbon was not limited, plant cell wall glycans and especially pectin, facilitated expansion of terrestrial flavobacterial strains over solid surfaces. While the specific correlation between expansion on pectin and the unique flavobacterial gliding motility mechanism was not determined in this study, it is evident that the latter is required, because ΔgldJ mutants did not expand on pectin. Propagation on pectin occurred in a concentration dependent manner, and the response was specifically dependent on the mother compound, as the response did not occur with bacteria grown on the pectin monomers D-galacturonic acid and L-

rhamnose. In contrast, growth on glucose was found to inhibit propagation of flavobacteria. This is supported by previous studies, which demonstrated that specific sugars such as glucose, sucrose and lactose can reversibly inhibit *F. johnsoniae* growth on minimal media and inhibits creation of raft microstructures, despite previous studies showing that glucose can served as a sole carbon source, and that it does not prevent gliding (47).

Patterns of *F. johnsoniae* colony expansion was dictated by the carbon source supplemented to the growth media. Preferable expansion of Flavobacteria occurred on plant-derived polysaccharides (pectin and glucomanan) creating a patchy pattern without uniform and dense coverage. The rapid expansion of flavobacteria on pectin was strongly linked to upregulation of TonB-related proteins, which undoubtedly assist in binding (recognition), uptake and metabolism of this glucan. Therefore, we hypothesize that for root-associated flavobacteria, pectin is not merely a carbon source but also an environmental cue. To the best of our knowledge, this is the first time that a specific environmental factor was found to be specifically linked to flavobacteria colony growth.

There are only few examples of plant cell wall-associated polysaccharides that facilitate root bacteria colonization. Flexibacter FS-1 is not able to glide on agarose but can glide on 1% Pectin, while galactronic acid does not induce similar effect (57). Purified Arabidopsis polysaccharides (arabinogalactan, pectin, or xylan) triggered biofilm and pellicle formation in *B. subtilis* when added to rich media and induced root colonization (6). Similarly, amendment with the PGPR *Bacillus amyloliquefaciens* strain SQY 162 with pectin and sucrose, increased bacterial abundance, induced biofilm formation and improved the ability to suppress tobacco bacterial wilt disease (58). In the symbiotic

nitrogen fixing bacterium *Rhizobium leguminosarum*, glucomannan-mediated attachment was important for legume infection and nodulation (12).

We did not observe any evidence of chemotactic behavior towards pectin, and therefore postulate that propagation on pectin requires direct contact between the flavobacteria and the glycan. Based on proteomics and subsequent gene expression validation using qPCR, we believe that this phenomenon is dictated by the induction of TonB-associated *F. johnsoniae* PULs, suggesting a link between niche recognition, colony expansion and metabolic fitness. Similar induction of TonB and PUL was observed in marine flavobacteria as response to phytoplankton blooms characterized in decomposition of alga-derived organic matter (59).

Their high energetic cost and substrate specificity of TonB transducers explains why they were induced by pectin and not constitutively expressed (60, 61). Nonetheless, the specific regulatory mechanism responsible for facilitating this response and the subsequent linkage to the induced gliding motility in flavobacteria remain an enigma. This is especially true in view of the fact that pectin did not induce expression of RemA, the lectin binding, flavobacterial cell surface adhesin that is a fundamental part of the gliding machinery (62). The gliding machinery might be involved in the earlier phase of the response to pectin, in which we observed intensive bacterial motility. This assumption can be examined by analysis of earlier proteomic profiles in the future.

Previous experiments showed that *Pseudomonas putida* mutants that lacked TonB, were deficient in their capacity to uptake iron and displayed impaired seed colonization, again linking TonB to metabolic and functional fitness (63). However, knock-out of specific TonB genes might be less effective in *F. johnsoniae* due to the multitude of predicted TonB genes in its genome, which suggests a high level of functional

redundancy (43). Similar functional redundancy of putative cellulolytic activity was shown where multiple cellulolytic enzymes were induced in proteomic analysis of the soil bacterium *Cytophaga hutchinsonii* after growth on cellulose (64).

In *Xanthomonas campestris pv. Campestris* pectate sensed by specific TonB-dependent receptor triggered secretion of extracellular polygalacturonate, resulting in pectin degradation and generation of oligogalacturonides (OGA) that are recognized as damage-associated molecular patterns (DAMPs), resulting in the initiation of the plant defensive measures (65). In light of our results and the findings in *X. campestris*, we speculate that sensing, uptake and degradation of pectin by TonB and associated proteins, results in OGA release, and plant immune response activation. This pathway may explain the biocontrol and improved plant disease resistance induced by flavobacteria in various plant types. Further experiments will be needed to support these assumptions.

Beside TonB related proteins, pectin induced additional protein targets (**Table S1**). The up regulation of auxin-regulated protein is especially interesting since Auxin is a major phytohormone responsible for plant growth and development demonstrating again a possible connection between pectin sensing and flavobacterial-plant interactions. The additional upregulation of putative transcriptional regulator may suggest pectin induce down-stream response.

Gene expression (qPCR) analysis did not completely mimic the expression differences found in the proteomics analysis of pectin vs. DDW. The discrepancy between RNA and protein expression might be explained by post-translational modifications or regulation affecting protein stability, degradation and complex formation in bacteria grown on pectin vs. DDW as shown in similar cases (66, 67).

Live-imaging fluorescent microscopy revealed that *F. johnsoniae* growth on solid surfaces was primarily bi-phasic in nature, with an initial phase of rapid expansion, followed by biomass production within the colonized area. It appeared that pectin initially induced colony expansion at the expense of cell growth. This bi-phasic growth pattern resembles previously described models in motile *E. coli*, which depicted an initial expansion phase, where "pioneer" bacteria with high velocity advance in front of the colony, followed by a second phase, where "settler" bacteria grow and replicate locally (16, 68). Interestingly, this bi-phasic phenomenon was most pronounced on pectin, suggesting that it may serve as signal that facilitate the expansion of "pioneer" cells, and later as carbon sources that support proliferation of "settlers".

To summarize, we found that pectin, a prominent plant cell wall polysaccharide, facilitates expansion of flavobacteria on solid surfaces, even in the presence of nutrient-rich media. We postulate that pectin may be a "missing link" in elucidating the mechanisms responsible for rhizosphere competence in flavobacteria, *i.e.* their capacity to efficiently colonize and proliferate in the root niche. Thus, in the root environment, plant cell wall polysaccharides, and specifically pectin, not only serve as a nutrient source for flavobacteria, but also as a cue for colonization and rapid expansion along the root surface. The interaction between pectin (and potentially other root glycans) and flavobacteria is mediated by induction of TonB/SusC operons and other associated PULs that facilitate propagation and metabolism of pectin.

Materials and Methods

Bacterial Strains and growth conditions

Four flavobacterial strains were targeted in this study. *F. johnsoniae* strain UW101 (ATCC17061), a gliding/secretion impaired *F. johnsoniae* strain UW101 gldJ mutant,

Flavobacterium sp. strain F52, isolated from the roots of a greenhouse pepper (69, 70)a gliding/secretion impaired *F52* strain. *F. johnsoniae* UW101 WT+pAS43 (Flavo GFP) Flavo-ErytRFlavo-CefR, *F. johnsoniae* UW102-48 (ΔgldJ)+pAS43 (Flavo GFP) Flavo-ErytR Flavo-CefR were used for microscopic analysis. Erythromycin, 100ug/ml was added to the media of the GFP labeled bacteria.

Flavobacteria strains were grown in CYE medium (Casitone, 10 mg/ml, yeast extract at 5mg/ml, and 8 mM $MgSO_4$ in 10 mM Tris buffer [pH 7.6]) at 30°C, as previously described (71). To observe colony-spreading, bacteria were grown on PY2 agar medium (2 g peptone, 0.5 g yeast extract,10 mg/ml agar, pH 7.3) at 30°C (72).

Organic amendments to growth media

A suite of sugars and other organic compounds were amended to growth media in various configurations (as described below), to evaluate motility and growth dynamics of the selected flavobacterial strains. The following substances were used in this study: pectin (Sigma P9135), arabinose (Sigma A9462), D(+)glucose (Merck K13506942), cellulose (Merck K239731), D(-)arbinose (Acros 161451000), , glucomannan (Megazyme P-GLCML), chitin (Sigma C7170), L-rhamnoae (Sigma 83650), D(+)-galacturonic acid monohydrate (Sigma 48280-5G-F),. All substances were dissolved to 2% final concentration in DDW. Glucose, arabinose, rhamnose and galactronic acid (titrated to pH 7) were dissolved and filtered through a0.22-micron filter. Pectin was dissolved in DDW heated at 80°C, and subsequently filtered on 0.45-micron filters. Chitin, cellulose, and glucomannan were dissolved in DDW heated to 50°C and then autoclaved for 20 min.

Flavobacteria growth on various sugars

The selected sugars were amended to the PY2 agar plates in two manners: (i) 10 µl of the selected compounds were thinly smeared along a line projecting outward from the center of the petri dish using a pipetor, to test the effect of specific carbon sources on the directional proliferation of flavobacteria (**Fig2S**) or (ii) 500µl were uniformly smeared over the entire petri dish to test the effect of specific carbon sources on the general proliferation of flavobacteria (**Fig1A, C, Fig2A**). Non-soluble substances such as cellulose and chitin, were vigorously vortexed and dispensed using a cut wide tip. Where two sugars were used (*i.e.* rhamnose+galactronic acid) we added 250µl of each. In all cases, plates were left to dry for overnight after adding organic amendments. Flavobacteria were thawed on CYE media overnight. Then bacteria were diluted in 200ul saline to 0.6-1 OD ($5x10^9$-$1.12\ x10^{10}$ cells), vortexed well and 2ul were spotted in the center of PY2 plate covered or streaked with specific sugar/s as indicated above. Plate was left to dry 15min and then grown for 48 hours in 30°C. The colony area or the length of expansion (in cm) were measured using Fiji (73) and statistics was calculated using JMP®, Version Pro 14 . (SAS Institute Inc., Cary, NC, 1989-2019). Differences between length/area were considered as significantly different when $p<0.05$ in Tukey HSD test unless indicated differently.

RNA extraction

To assess the expression of selected genes in the presence of pectin, total RNA was extracted from *F. johnsoniae* cells grown for 48hours at 30°C on PY2 plates covered with 500µl of DDW or 2% pectin as described above. For each plate, bacteria were suspended in 1 ml cold (4°C) PBS buffer, washed once in cold PBS, centrifuged for 2min at 18,000g , re-suspended in TE buffer supplemented with 0.4mg/ml Lysozyme and then incubated in 10min in room temprature. RNA was subsequently extracted from cells using the TRIzol reagent (TRIzol® InvitrogenTM, #15596026), following the

manufacturer's instructions. Residual DNA was removed from the RNA samples by digesting with RQ1 DNAse (Promega M6101A) at 37 °C for 40 min. For the real time experiments, cDNA was synthesized using 50ng of DNAse treated RNA, with 1ul of random prokaryotic primers (Promega C118A). Synthesis of single strand cDNA was achieved using ImProm-IITM Reverse-Transcriptase (Promega, Madison, WI, United States).

The integrity and concentration of the extracted RNA and cDNA, was examined with a QubitTM 3.0 Fluorometer (Thermo Fisher Scientific, United States) using reagents and protocols supplied by the manufacturer and electrophoresis of samples on a 0.8% agarose gel.

Live Imaging Fluorescent Microscopy Experiments.

To visualize the effect of different plant derived sugars on *F. johnsoniae*, we prepare a 24 well plate with 500µl PY2 agar media in each well. 10ul of each examined 2% substance was gently applicated on the well in triplicates, and plate was rotated for 1hour and dried ON in RT. A 30G needle (KDL 16-0310) was used to seed the bacteria in the center of each well. Microscopic imaging was performed using a NIKON eclipse Ti microscope (Nikon, Japan) equipped with a ProScan motorized XY stage and an HF110A system (enabling rapid switching of emission filters)(Prior Scientific, MA, USA), and a temperature-controlled stage incubator (LAUDA ECO RE 415, Korea). Bright field illumination was provided by a cool LED pE-100A (Cool LED, UK). Excitation light for epifluorescence microscopy was provided by a Spectra X light engine (Lumencor, USA). Imaging was performed using a long working distance 40x objective (NA 0.6) (Nikon, Japan). Images were captured at 2 hours intervals for 44 hours using an ANDOR zyla 5.5 MP sCMOS camera (Oxford Instruments, UK). For

each frame, bright field (gray), phycocyanin (Ex: 555 nm/Em: 632 nm; pink) and chlorophyll (Ex: 440 nm/Em: 697 nm; green) were captured separately. Images were processed using the NIS elements AR 4.6 (64 bit) software package (Nikon, Japan) and Fiji (73). Fluorescence level was normalized to the initial measured value (to avoid differences in the initial number of seeded bacteria) and to the maximal fluorescence on DDW (to reduce variability of GFP fluorescence levels between movies). The population growth dynamic of GFP- *F. johnsoniae* on each substance computed using Fiji's time series analyzer plugin, and average fluorescence density profiles of the expanding population over time was quantified using JMP®, Version Pro 14 . (SAS Institute Inc., Cary, NC, 1989-2019).

Proteomics analysis

The samples were subjected to in-solution tryptic digestion followed by a desalting step. The resulting peptides were analyzed using nanoflow liquid chromatography (nanoAcquity) coupled to high resolution, high mass accuracy mass spectrometry (Q Exactive HF). Each sample was analyzed on the instrument separately in a random order in discovery mode. Raw data was searched against the *F. johnsoniae* protein databases, to which a list of common lab contaminants was added.

Database search was done using the Mascot algorithm, and quantitative analysis was performed using the Expressionist software from GeneData. As a rule of thumb we consider significant differences to be >1 peptide per protein, fold change >2 or <0.5, and <0.05 p-value. Proteins were functionally annotated using RAST (Rapid Annotations using Subsystems Technology) (74).

Twenty-five pectin-induced proteins were selected based on high fold change between pectin vs. DDW and statistical significance (proteins without annotation were removed

from this analysis). Each gene abundance across the dataset were normalized to 100% and a heatmap was created using studio.plot.ly (https://plotly.com/).

Quantitative PCR Assessment of Gene Expression Levels

The expression of 10 genes encoding for proteins found to be significantly induced on pectin in the proteomic analyses were analyzed using quantitative realtime (qRT)-PCR. For relative quantification of target genes, primers for qRT-PCR experiments (**Table S5**) were based on the *F. johnsoniae* genome sequence and were predesigned using the PrimerQuest® tool (Integrated DNA Technologies -IDT). Triplicate cDNA samples for each of the treatments (Pectin/DDW) were diluted and 2ng was used in a 20 µl final reaction volume together with 10ul Fast SYBR™ (Thermo Fisher scientific) green PCR master mix, 100 nM each forward and reverse primers, DDW and 1ul of template cDNA. Amplification was carried out on a StepOnePlus real-time PCR thermocycler using the following program: heat activation of DNA polymerase at 95°C for 3 min and 40 cycles at 95°C for 5 sec for denaturation and primer annealing and extension at 60°C for 30 sec. A melt curve was produced to confirm a single gene-specific peak and to detect primer-dimer formation by heating the samples from 60 to 95°C in 0.3°C increments. Gene amplification was confirmed by detection of a single peak in the melt curve analysis. No primer-dimer formation was detected. For each gene, PCR gene amplification was carried out with three independent biological replicates. The relative quantification of each target gene versus reference gene was determined according to the method described (Livak and Schmittgen2001). Expression of each gene was normalized to that of three alternative reference genes (16S rRNA, DNA gyrase subunit B (EC 5.99.1.3) -*gyr*B (Housekeeping), and Electron transfer Flavoprotein, alpha subunit, Threonine synthase (EC 4.2.3.1)-ETF), selected because there was no detected difference in its expression between the pectin and DDW treatments in the proteomic

analysis. Concentrations and ΔΔCT values were calculated and analyzed with the StepOne software v2.3 (Applied Biosystems, Foster City, CA, United States). Concomitant "no-RT" reactions, lacking reverse transcriptase, were performed for each sample and run to confirm absence of DNA contamination, as well as no template controls (NTCs) to confirm lack of contamination of all used reagents. Efficiency of reactions was monitored by means of an in internal standard curve using a 10-fold dilution of DNA ranging from 0.01-10ng of DNA per reaction, done in triplicates. Reported efficiency was between 92.1 and 97.2% for all primers, and R2-values were greater than 0.99. All runs were performed using a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, United States) and data analysis was conducted using the StepOne software v2.3 (Applied Biosystems, Foster City, CA, United States).

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Figures legend

Fig1- Impact of sugars on proliferation of flavobacteria. Image (A) and quantification (B) of colony area of Flavobacteria colony expansion on PY2 agar amended on different mono- and polysaccharides after 48hr incubation at 30°C (N=4). Image (C) and quantification (D) of colony expansion on PY2 agar of wild type (WT) and gliding mutants (ΔgldJ) of *F. johnsoniae* and *Flavobacterium* sp. F52, amended with DDW or 2% pectin after 48hr incubation at 30°C (N=3). Colony area was measured using Fiji. Statistics significance calculated using ®JMP Pro14, was considered significant if $p<0.05$ by Tukey HSD.

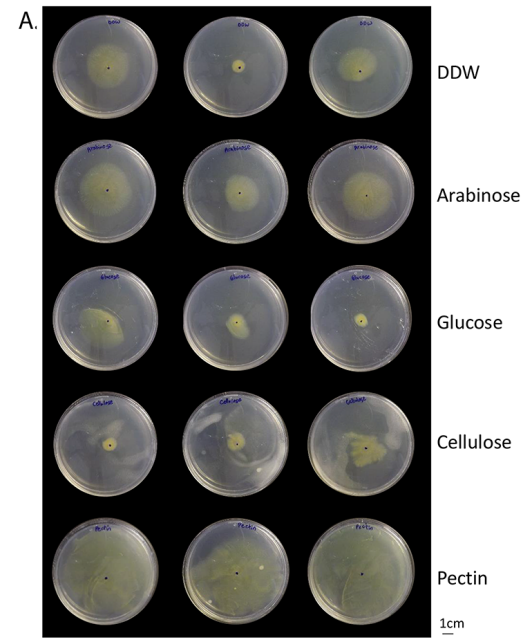
Fig2 - Impact of pectin precursors (galactronic acid and rhamnose) on proliferation of flavobacteria. (A) *F. johnsoniae* colony expansion on PY2 agar amended with DDW, pectin galactronic acid, rhamnose and galactronic acid and rhamnose incubated at 30°C for 48 h (N=4). (B) Graphic description of colony area based on results from (A). Statistics significance was calculated using ®JMP Pro14, and means was considered significant when $p<0.05$ by Tukey HSD

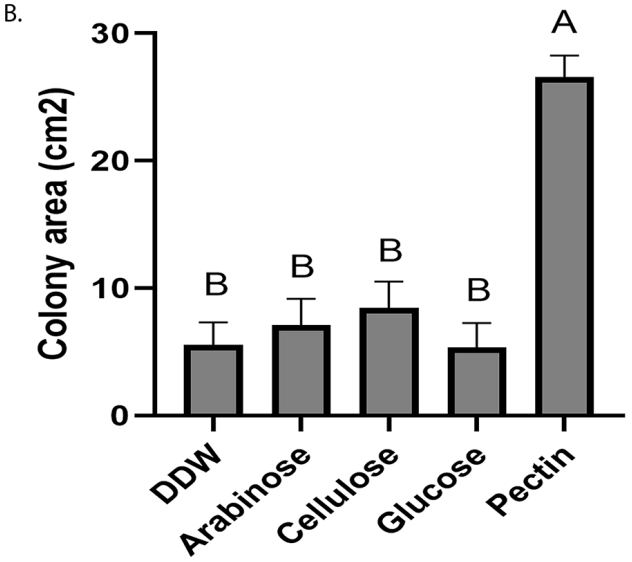
Fig3 - Temporal dynamics of flavobacterial proliferation on different sugars using live imaging microscopy. (A) Morphology of GFP labeled *F. johnsoniae* colonies on selected sugars. Bacteria were inoculated in the center of PY2 agar coated with the indicated sugars (schematically described in the insert). Images show colony morphology after 32hr. (B) Enlarged image of GFP-*F. johnsoniae* colony morphology after 20hr of growth on the selected sugars as indicated in (A). (C) Growth rates of GFP-*F. johnsoniae* colonies on the selected sugars. Data was normalized as described in the materials and methods section. Differences in the average colony fluorescence

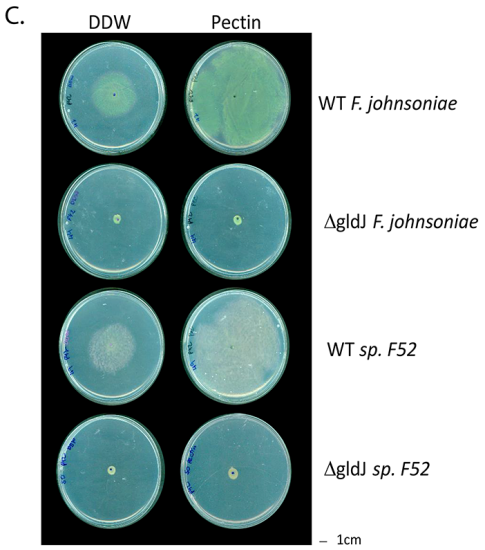
intensity after 44 hr was compared and considered significant if $p<0.05$ by Tukey HSD. Data includes means and data from three biological replicates composed of three technical repeats in each. (D) Temporal dynamics of GFP-*F. johnsoniae* growth rates. Growth was compared at the peaks (20hr and 36hr) and considered significant if $p<0.05$ by Tukey HSD. (E) Schematic diagram showing the three characterized regions of interest (ROI- 1.5, 3 and 4.5 mm radii) used to evaluate of bacterial expansion rates. (F) Estimated expansion rates of GFP-*F. johnsoniae* on the selected sugars. Velocity of bacterial movement was estimated by expansion time in hours taken to cross known ROIs as indicated in E. Statistical significance is $p<0.05$ by Tukey HSD. (G) Estimated expansion time relative to estimated growth of GFP-*F. johnsoniae* on the selected sugars. Colored circles mark time (h) for bacteria to cross the 4.5mm radius on each substance as calculated in F.

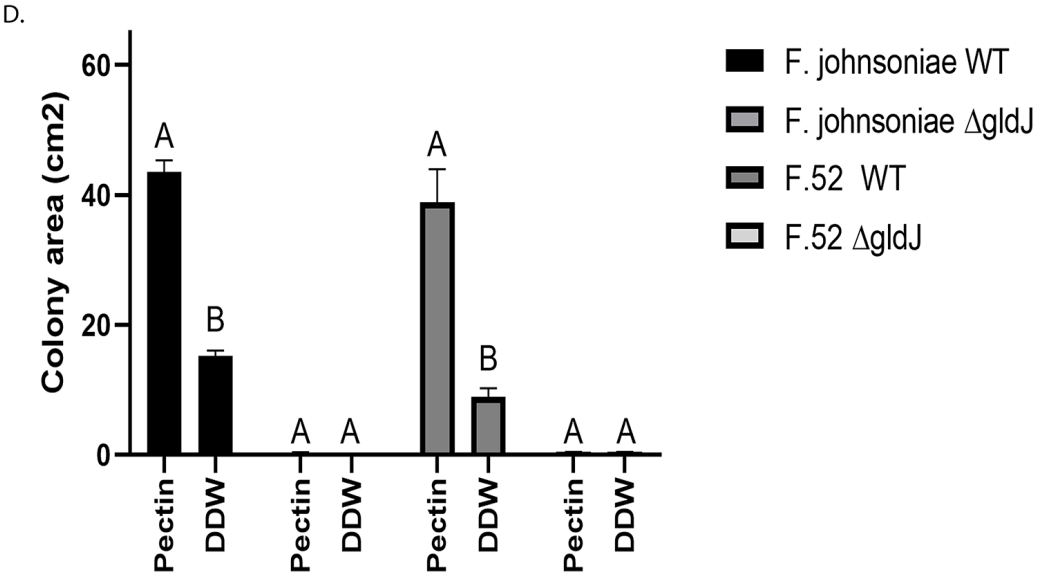
Fig4 - Pectin induced flavobacterial genes and proteins. (A) Differential expression of the 25 most substantial pectin induced proteins based on proteomic analysis of *F. johnsoniae* colonies grown on CYE medium amended with pectin relative to colonies grown on identical media amended with DDW. Heat map shows triplicates for each treatment. All described proteins are statistically significant ($p<0.05$). (B) Expression of selected genes (sus73, tonB260, tonB445, tonB 2144, auxin regulator, Transcriptional regulator, pectate lyase and remA) shown to be induced in the proteomic analysis described in (A), using quantitative real-time PCR (qPCR). Fold changes in mRNA levels of the target genes were normalized against the 16SrRNA gene (left), the Electron transfer Flavoprotein, alpha subunit (center), and the DNA gyrase subunit B (right). Change in target genes fold change RNA expression was calculated using the $2^{-\Delta\Delta CT}$ method and statistical significance ($p<0.05$) by Student T-

test. Error bars represent standard errors of six independent experiments based on two independent RNA extractions.

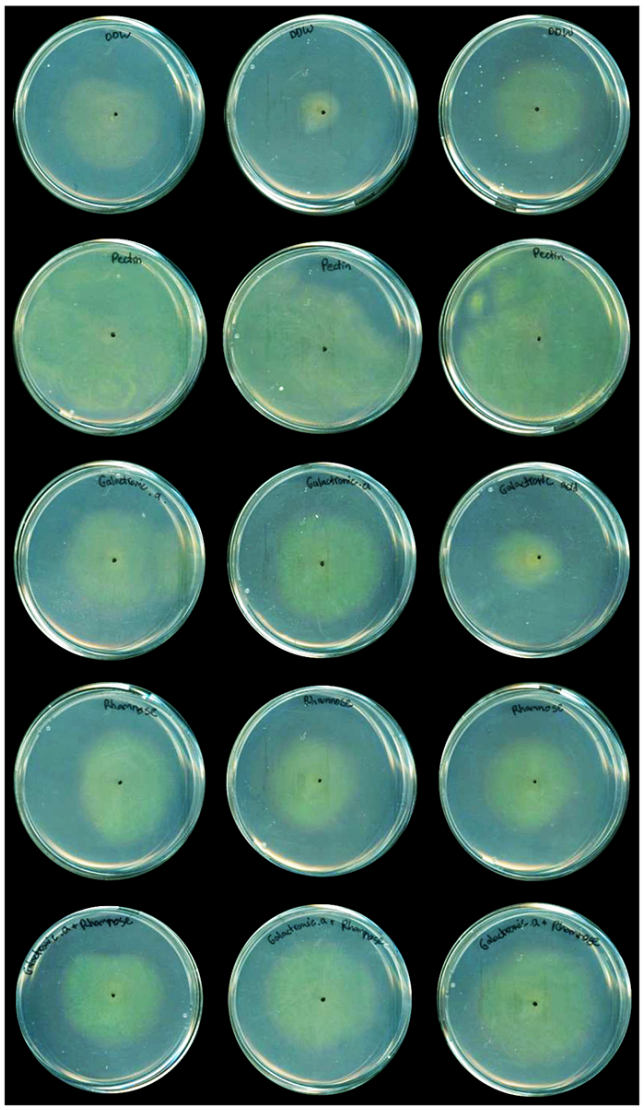

A.
DDW
Arabinose
Glucose
Cellulose
Pectin
1cm


B.
Colony area (cm2)
30
20
10
0
A
B
B
B
B
DDW
Arabinose
Cellulose
Glucose
Pectin

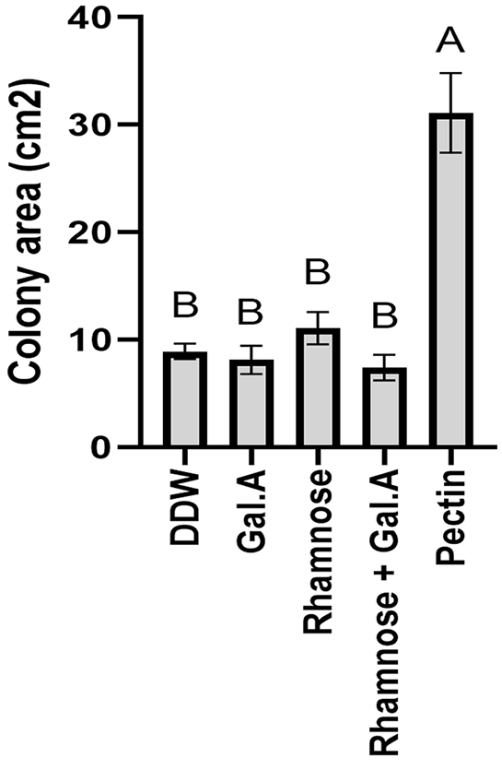

C.
DDW
Pectin
WT F. johnsoniae
ΔgldJ F. johnsoniae
WT sp. F52
ΔgldJ sp. F52
1cm

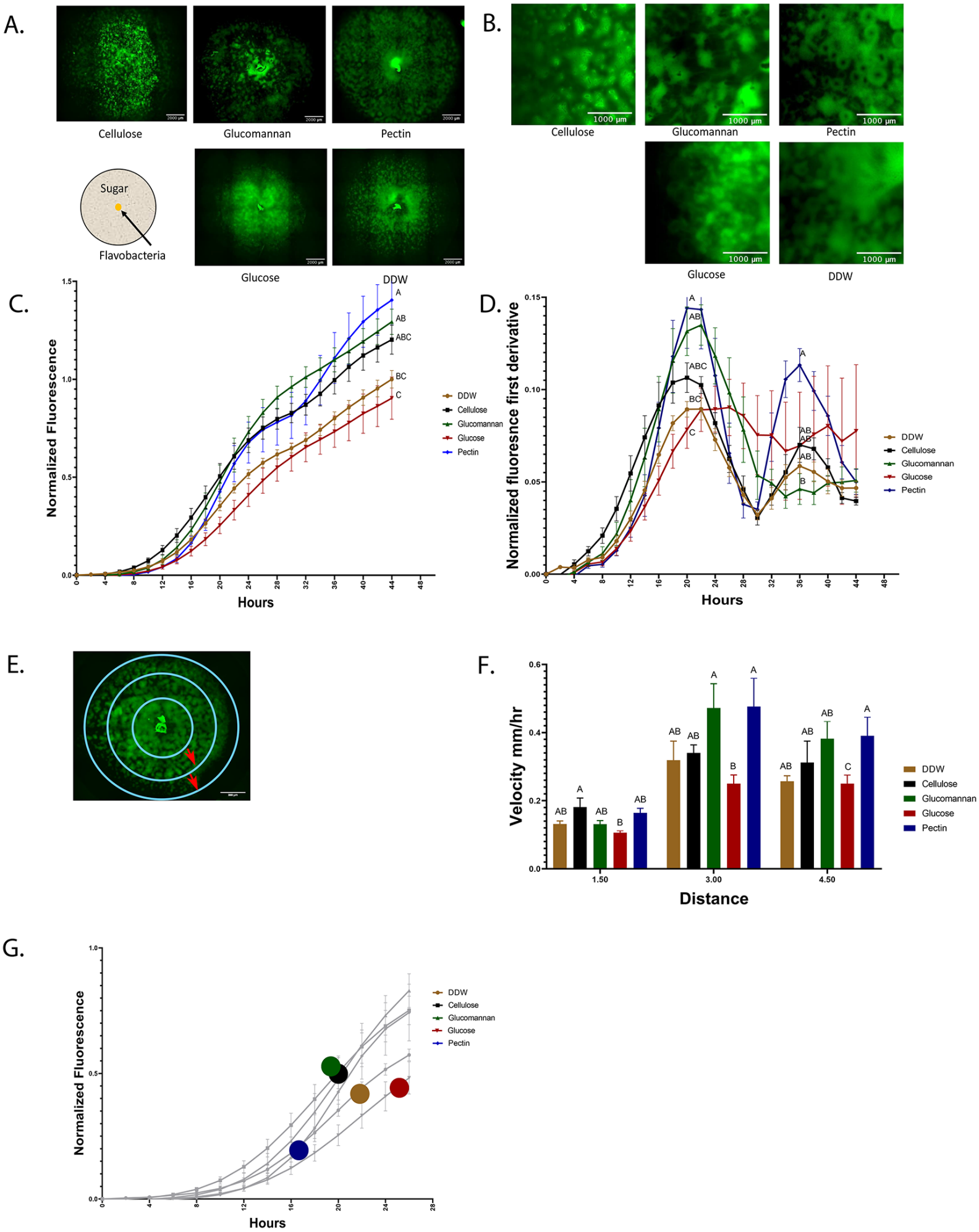

D.
Colony area (cm2)
60
40
20
0
A
B
A
A
A
B
A
A
Pectin
DDW
Pectin
DDW
Pectin
DDW
Pectin
DDW
F. johnsoniae WT
F. johnsoniae ΔgldJ
F.52 WT
F.52 ΔgldJ

A.

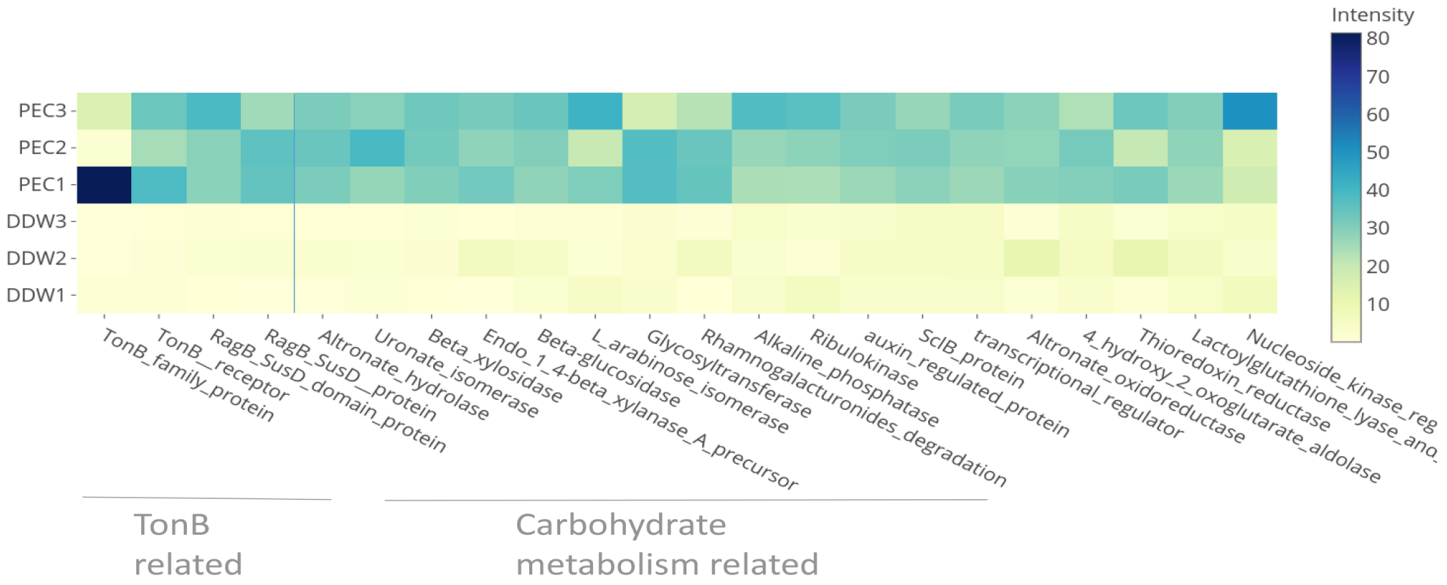

DDW
Pectin
Galacturonic acid
Rhamnose
Rhamnose
+ Galacturonic acid
1 cm

B.


Colony area (cm2)
40
30
20
10
0
B
B
B
B
A
DDW
Gal.A
Rhamnose
Rhamnose + Gal.A
Pectin


A.
Cellulose
Glucomannan
Pectin
Sugar
Flavobacteria
Glucose
B.
Cellulose
Glucomannan
Pectin
Glucose
DDW
C.
Normalized Fluorescence
Hours
DDW
Cellulose
Glucomannan
Glucose
Pectin
D.
Normalized fluoresnce first derivative
Hours
E.
F.
Velocity mm/hr
Distance
G.
Normalized Fluorescence
Hours

A.


Intensity
80
70
60
50
40
30
20
10
PEC3
PEC2
PEC1
DDW3
DDW2
DDW1
TonB_family_protein
TonB_receptor
RagB_SusD_domain_protein
RagB_SusD_protein
Altronate_hydrolase
Uronate_isomerase
Beta_xylosidase
Endo_1_4-beta_xylanase_A_precursor
Beta-glucosidase
L_arabinose_isomerase
Glycosyltransferase
Rhamnogalacturonides_degradation
Alkaline_phosphatase
Ribulokinase
auxin_regulated_protein
ScIB_protein
transcriptional_regulator
Altronate_oxidoreductase
4_hydroxy_2_oxoglutarate_aldolase
Thioredoxin_reductase
Lactoylglutathione_lyase_ano
Nucleoside_kinase_reg
TonB related
Carbohydrate metabolism related

B.

Sus73
TonB260
TonB445
TonB2144
Pec Lyase
RemA
Auxin Regul.
Trans. Regul
A
AB
BC
C
C
C
C
C
A
A
B
B
B
B
B
B
A
A
B
B
B
B
B
B
0
50
100
150
200
250
300
350
400
450
500