Investigation of potassium tetraborate resistance in *Dickeya* spp.

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Abstract

Dickeya spp. are common plant pathogens associated with bacterial soft rot, potato blackleg, and slow wilt, which are plant diseases that account for major losses in the agricultural industry. The diseases caused by these bacterial species are not yet fully managed with existing techniques, and new approaches need to be considered to minimize future crop loss. Previous research has shown that the inorganic salt potassium tetraborate tetrahydrate (PTB) can inhibit the growth of *Dickeya* species; however, disk diffusion assays result in a unique phenotype with two zones of inhibition. This study investigates the effects of PTB on the growth of four *Dickeya* spp.. It was hypothesized that the production of phage is responsible for the two zones of inhibition. Disk diffusion assays and growth curves were used to confirm the impact of PTB on *Dickeya* and attempts were made to directly isolate phage from the strains. To elucidate the mechanism of action of PTB, Tn-Seq libraries were used to determine which genes are required for growth in the presence of PTB. Tn-Seq libraries showed that different *Dickeya* strains shared seven overlapping genes including stress-related genes that increase bacterial resistance to PTB. Gene expression studies were used to determine the changes in gene expression that result from PTB exposure. Preliminary results showed that exposure to PTB induces the expression of stress-related genes in *Dickeya* to increase survival in the presence of the compound. Further research is needed to better understand the implications of observed changes in bacterial gene expression.

Introduction

Members of the *Pectobacteriaceae*, a family of Gram-negative bacteria, are known to cause bacterial soft rot and potato blackleg among other diseases which are destructive to many crops in the fields (Charkowski, 2018; Toth et al., 2021; Toth et al., 2011). It has been reported that bacterial soft rot losses after crop harvest account for up to a third of total loss, significantly greater than any other disease (Agrios, 2005). The severe losses in agriculture yield from soft-rot plant pathogens make the study of these soft-rot pathogens crucial to the success of the agricultural industry. Soft-rot diseases are particularly detrimental to potatoes, the fourth most important crop in the world, causing up to two-thirds of losses (Mantsebo et al., 2014). Soft-rot diseases are currently largely managed by good agricultural and handling practices, but better prevention mechanisms are needed to sustain the food needs of a growing population (Olsen & Nolte, 2011). Although there are currently several management options used to control the bacteria, these options are limited, and alternative methods need to be considered. Existing management options include cultivation measures and hygienic practices such as using clean seed and planting in pathogen-free areas, optimizing storage conditions to decrease the development and spread of disease, physical seed treatments with heat and UV radiation, and chemical treatments primarily with antibiotics (van der Wolf et al., 2021). Other chemical treatment options to prevent infection are being explored, including the use of inorganic salts. It has been reported that inorganic salts inhibit the growth of Pectobacterium, a genus within *Pectobacteriaceae*, the same family as *Dickeya* (Yaganza et al., 2009). Not only

does the agricultural industry seek at inhibiting growth of *Dickeya*. An interesting double-ring-of-inhibition phenotype was observed with *Dickeya* in diffusion assays using agar plates (Liu & Filiatrault, 2020). This phenotype has not been previously seen in *Dickeya* or other bacteria. This study explored many questions pertaining to *Dickeya*'s unique response to PTB and PTB's mechanism of action. PTB can be considered for further investigation as a potential chemical treatment option for crops in storage and fields if it is found to be an effective inhibitor of soft rot diseases.

The mechanism of action of PTB is not yet fully known but understanding how the chemical affects bacterial growth is crucial to uncovering its potential for agricultural use. Previous research found that soft-rot *Pectobacteriaceae* including both Pectobacterium and *Dickeya* may have phages (Czajkowski, 2016; Adriaenssens et al., 2012; Resibois et al., 1984) and phage-like elements (Czajkowski, 2019) in their genome that contribute to bacterial virulence. The presence of prophages can be extremely beneficial to bacterial fitness and can introduce new traits into the host (Bondy-Denomy & Davidson, 2014; Nanda et al., 2015). These new traits can alter the bacterial genome in many ways, one of which is by changing the bacteria's pathogenicity and responses to stress (Hacker & Carniel, 2001; Fortier & Sekulovic, 2013; Casjens, 2003). Stress conditions can cause phage induction and kill the bacterial host (Du Toit, 2019), and compounds containing boron such as PTB have been found to increase oxidative stress in pathogens (Qin et al., 2010). Although the details are not well understood, phage present in plant-pathogenic bacteria can carry genes that alter plant pathogenicity (Varani et al., 2013).

The main objective of our research was to investigate the effect of PTB on growth and gene expression in *Dickeya* spp.. Possible reasons for the unique disk diffusion phenotype were explored by testing a larger collection of *Dickeya* strains and exploring four hypotheses tailored to possible reasons for the phenotype displayed by *Dickeya* when exposed to PTB.

Hypothesis 1: The inner ring represents bacteria that are resistant to PTB.

Hypothesis 2: The zone of clearing is the result of phage-induced gene expression.

Hypothesis 3: The genes responsible for sensitivity or resistance in *Dickeya* are different from those found in Pectobacterium.

Hypothesis 4: The presence of PTB induces production of phage by *Dickeya* spp..

Methods and Materials

Bacterial Strains

Dickeya dadantii 3937, *Dickeya dianthicola* ME23, *Dickeya dianthicola* 67-19 and *Dickeya fangzhongdai* 643-a were cultured on lysogeny broth (LB) agar plates or in LB broth (10 g tryptone, 5 g yeast extract, and 10 g NaCl per 1 L) at 28 °C (Bertani, 1951).

Antibacterial Activity of PTB

Disk diffusion assays were used to test the antimicrobial activity of PTB on the four strains of *Dickeya* and to confirm their double-ring phenotype (Liu & Filiatrault, 2020). Triplicate overnight cultures were grown in LB, adjusted to 2×10^8 c.f.u. ml^{-1} (OD_{600} = 0.2), and split into three replicates. The cultures were spread on LB agar plates using sterile cotton swabs, and one sterile paper filter disk (6 mm in diameter; GE Healthcare) loaded with 20 µl filtered 500 mM PTB solution was put on each plate. Plates were incubated at 28°C for 24 hours after which pictures were taken.

Direct Phage Isolation

Several different protocols were used in our attempts to isolate phage from the bacteria in the double zone (Figure 1) and bacteria were grown in liquid culture with PTB. These protocols included a phage genomic DNA extraction (modified Promega Wizard method; Promega, Madison, WI, USA) as well as several adaptations involving chloroform (*Phage genomic DNA extraction*, 2011; *Protocol for Phage DNA Extraction*, 2018; Adams, 1959).

Growth Curve

Growth curves were performed using a Synergy 2 multi-mode microplate reader (Liu & Filiatrault, 2020). Overnight cultures of the four strains of *Dickeya* were grown in LB and adjusted to 2×10^8 c.f.u. ml^{-1} (OD_{600} = 0.2). 200 µl of adjusted overnight culture with PTB concentrations of 0 mM, 1.88 mM, 3.75 mM, 7.5 mM, and 15 mM were measured at approximately 25 °C in for up to 16 h at 30 min intervals. Concentrations were chosen based on growth curves performed on Pectobacterium (Liu & Filiatrault, 2020). Six technical replicates and three biological replicates were performed for each treatment. The vertical lines represent the standard error of the mean.

Tn-Seq Libraries

Previously constructed randomly barcoded Tn-Seq libraries from Helmann et al. (2022) were used to identify genes of interest in three strains of *Dickeya*: *D. dadantii* 3937, *D. dianthicola* ME23, and *D. dianthicola* 67-19. The libraries were recovered in LB with kanamycin and incubated at 28 °C for 7 hours until reaching an OD600 of 0.5-0.7 and split into four replicates. 24-well plates were prepared with LB and PTB concentrations of 0 mM, 1.88 mM, 3.75 mM, and 7.5 mM. The libraries were inoculated in the 24-well plates and grown overnight. Genomic DNA was extracted using the Monarch Genomic DNA Purification Kit (New England Biolabs, Ipswich, MA, USA). Helmann performed the PCR on the extracted DNA to amplify the barcode sequence and index each sample, and then pooled all the samples to submit to the Biotechnology Resource Center (BRC) Genomics Facility at the Cornell Institute of Biotechnology for Illumina sequencing. The libraries had been previously mapped (Helmann et al., 2022). Tn-Seq mapping data was analyzed using the FEBA RB-TnSeq analysis pipeline to identify genes for each strain that are important for growth in PTB (Helmann et al., 2022; Wetmore et al., 2015).

Real-Time Quantitative PCR

Three strains of *Dickeya*, *D. dadantii* 3937, *D. dianthicola* ME23, and *D. dianthicola* 67-19, were grown overnight. The next day, half of the bacteria were exposed to 3.75 mM PTB and cultured for another hour while the other half also were cultured for another hour without PTB. The PTB concentration used was selected based on growth curve results. RNA from bacteria grown with and without PTB were used in the qRT-PCR experiments to determine differences in gene expression. RNA was extracted using the Qiagen RNeasy kit (Hilden, Germany), treated with Ambion DNase I (Thermo Fisher, Waltham, MA, USA), and cleaned using the Zymo Clean and Concentrate kit (Irvine, CA, USA). cDNA was made from RNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA), and the resulting cDNA was quantified and diluted to 100 ng/µL. The SsoAdvanced Universal SYBR Green Supermix instruction manual (Bio-Rad) was used for real time PCR reactions. Five genes selected from Tn-Seq results were evaluated. For *D. dadantii* 3937, the genes were: Mannose-1-phosphate guanylyltransferase/mannose-6-phosphate isomerase, phosphomannomutase/phosphoglucomutase,GDP-mannose 4,6-dehydratase, WbeA, and sigma E protease regulator RseP. For *D. dianthicola* 67-19, the genes were: GDP-mannose 4,6-dehydratase, phosphomannomutase CpsG, mannose-1-phosphate guanylyltransferase/mannose-6-phosphate isomerase, envelope stress response regulator transcription factor CpxR, and microconductance mechanosensitive channel MscM. The reactions were set up with SYBR Green Supermix at 1x, 200 nM of each primer, and 100 ng of cDNA. The thermal cycling protocol was as follows: denaturation at 95 °C for 10 seconds, annealing and extension at 52 °C for 30 seconds, and cycled through 40 cycles on a Bio-Rad Connect. Due to time constraints, qRT-PCR was performed on RNA isolated from two strains: *D. dadantii* 3937 and *D. dianthicola* 67-19. Three technical replicates and three biological replicates were performed for each treatment. Data was analyzed using the delta delta Ct analysis (Bradburn, 2020), and the vertical lines represent the standard deviation of the mean.

Results

Antibacterial Activity of PTB

Disk diffusion assays were used to test the antimicrobial activity of PTB on the four strains of *Dickeya* to determine whether the phenotype was conserved among species and if colonies growing in the presence of PTB developed resistance. It was found that all strains demonstrated the double-ring phenotype, suggesting that the phenotype is conserved in multiple *Dickeya* species (Figure 1). To determine if the bacteria growing between the two zones of clearing are resistant, bacteria were isolated and used to repeat the same disk diffusion assay. The disk diffusion assay for bacteria taken from the area between the two zones (bacterial growth between red arrow, inner zone, and black arrow, outer zone) gave phenotypically similar results as bacteria not previously exposed to PTB (Figure 2). This assay ruled out the possibility PTB had led to the selection of PTB resistant bacteria and rejected hypothesis 1 – had there been evolution, the bacteria between the two zones would all be resistant to PTB in the disk diffusion assay. No colonies were found in the zone of clearing. Since the same phenotype with two zones of inhibition was observed even when using the bacteria grown between the two zones of the original assay, it was concluded that the bacteria between the two zones were still sensitive to PTB and that the PTB was not selecting for resistant bacteria.

Direct Phage Isolation

Attempts were made to directly isolate phage from all four *Dickeya* strains to test Hypothesis 2 and determine whether the presence of PTB induces production of phage by *Dickeya* spp.. A phage genomic DNA extraction (modified Promega Wizard method; Promega, Madison, WI, USA) and several adaptations (*Phage genomic DNA extraction*, 2011; *Protocol for Phage DNA Extraction*, 2018; Adams, 1959) were used, all of which failed to produce any sort of pellet after centrifugation. Phage isolation attempts were unsuccessful, and phage DNA was unable to be extracted using any of the protocols. Many variables were in play with direct phage isolation. It was possible that the phage was not released from the bacteria used, and gene expression experiments (qRT-PCR) and Tn-Seq were instead used to investigate phage presence.

Growth Curve

Growth curves were performed to determine the minimal inhibitory concentration (MIC) of PTB at which each *Dickeya* strain cannot grow. All strains grew in cultures lacking PTB, and all four *Dickeya* strains were found to be sensitive to PTB depending on the concentration (Figure 3). 15 mM PTB showed to completely inhibit the growth of *D. dadantii* 3937 and *D. fangzhongdai* 643-a while 7.5 mM PTB was sufficient to inhibit the growth of *D. dianthicola* ME23 and *D. dianthicola* 67-19 (Figure 3). While the double-ring phenotype of interest cannot be observed using growth curves, the MIC was used to guide the concentrations tested in the Tn-Seq experiments.

Tn-Seq Libraries

Tn-Seq libraries were used to identify genes in *Dickeya* important for growth in PTB. The genes identified were compared to genes found in PTB-resistant Pectobacterium (Liu & Filiatrault, 2020) and were used in qRT-PCR to evaluate changes in gene expression. The libraries revealed several genes that when disrupted improve growth in PTB and several genes that when disrupted are detrimental to growth in PTB (Table 1). All experiments passed established quality check parameters (Helmann et al., 2022; Wetmore et al., 2015) and the genes shown in Table 1 were all statistically significant with $|fit| > 1$ and $|t| > 4$. Normalized gene fitness (fit) values were used to sort the genes. A positive fit value would indicate that the strain is more resistant to PTB, and the disruption in the gene improved survival. A negative fit value would indicate that the strain is more sensitive to PTB, and the disruption in the gene was detrimental to the bacterial strain. In *D. dadantii* 3937, nine genes improved growth in PTB when disrupted, and 14 genes were detrimental to growth in PTB

when disrupted; in *D. dianthicola* ME23, six genes improved growth in PTB when disrupted, and nine genes were detrimental to growth in PTB when disrupted; in *D. dianthicola* 67-19, five genes improved growth in PTB when disrupted, and five genes were detrimental to growth in PTB when disrupted (Table 1). Seven genes were shared between at least two of the three strains tested including mannose-1 phosphate guanylyltransferase/mannose 6-phosphate isomerase, phosphomannomutase/phosphoglucomutase,GDP-mannose4,6-dehydratase, microconductance mechanosensitive channel MscM, and envelope stress response regulator transcription factor CpxR (Figure 4). The genes related to sensitivity and resistance in *Dickeya* are different from those found in Pectobacterium (Liu & Filiatrault, 2020) which confirms Hypothesis 3. *Dickeya* and Pectobacterium respond to PTB through different mechanisms – the genes found in *Dickeya* can then be used to study differences in gene expression and potential overlap with phage-related genes.

Real-Time Quantitative PCR

To track the differences in gene expression between PTB-exposed bacteria and non-exposed bacteria, qRT-PCR was performed using the genes that were identified using Tn-Seq (Table 1). Five genes were selected from the Tn-Seq experiments to use as targets for qRT-PCR, and the resulting data was analyzed using the delta delta Ct analysis (Bradburn, 2020). Due to time constraints, each experiment was run once and for only two strains of *Dickeya*. *D. dadantii* 3937 and *D. dianthicola* 67-19 varied greatly in gene expression. Unexpectedly, all five genes tested for *D. dianthicola* 67-19 had fold changes in gene expression < 1, which would mean that all five genes demonstrated reduced expression in PTB-treated samples (Figure 5). This result did not agree with our expectations since three of the genes tested are detrimental to growth in PTB when disrupted (Table 1).

Discussion

Dickeya displayed a unique response to PTB in disk diffusion assays by showing two distinct rings of clearing (Figure 1). The observed phenotype was hypothesized to potentially be the result of phage production as a response to PTB exposure. Previous research has found *Dickeya* to contain phages (Czajkowski, 2016; Adriaenssens et al., 2012; Resibois et al., 1984) and phage-like elements (Czajkowski, 2019) which could impact bacterial fitness when exposed to a stressor like PTB. All four strains reacted similarly in the disk diffusion assays and were sensitive to PTB in LB, which suggests that response to PTB may be a conserved mechanism.Through multiple disk diffusion assays and the Tn-Seq experiments, it was determined that PTB exposure did not select for resistant *Dickeya*, and the genes responsible for PTB sensitivity or resistance in *Dickeya* did not overlap with mutated Pectobacterium genes previously found (Liu & Filiatrault, 2020). Further research can be done to more closely investigate these differences.The Tn-Seq experiments provided important information about genes involved in *Dickeya* resistance and sensitivity to PTB. Tn-Seq experiments showed that *Dickeya* strains shared several genes of interest, many of which were stress-related genes that may help increase bacterial resistance to PTB. The double-ring phenotype could have been caused by changes in gene expression at different PTB concentrations, but more research is needed to draw conclusions. Stress-related genes are likely required for growth in PTB; borate has been shown to inhibit growth and induce stress responses of certain pathogens (He et al., 2019). Bacteria respond to stress in a variety of ways, which can include inducing of expression of different genes relating to survival and virulence. Changes in gene expression related to mannose-metabolism have been previously observed in pathogenic bacteria as a response to osmotic stress (Chowdhury et al., 1996). The disturbance of mannose-metabolism in response to salinity is consistent with the observed importance of mannose-related

genes for growth in PTB (Table 1). The genes disrupted in *Dickeya* were different than the mutated genes found through whole-genome sequencing of PTB-resistant Pectobacterium mutants which affected peptide chain release from the ribosome (Liu & Filiatrault, 2020). The difference between responses to PTB in *Dickeya* and Pectobacterium may indicate that even though both bacteria are part of the same family of *Pectobacteriaceae*, they may not react to compounds in the same way and PTB may be effective on one genus but not the other. Further research is necessary to elucidate the differences in response to PTB between the two genuses and to determine whether responses are consistent within different species of the genus. We hypothesized that phage was responsible for *Dickeya*'s response to PTB exposure but attempts to directly isolate phage were unsuccessful. Phage isolation can be heavily timing dependent – it is possible that our isolation attempts did not align with the timing of phage release or that phage was not released from the bacteria. Further research would be necessary to thoroughly explore phage-related responses of *Dickeya* to PTB exposure. No firm conclusions were made regarding phage production due to time constraints. If a suitable phage-sensitive host can be found, plaque assays can be used to identify phage plaques. Otherwise, continued exploration of gene expression would likely be the preferred route. The Tn-Seq experiments revealed genes related to lipopolysaccharide (LPS) production, which has been found to interact with phage (Andres et al., 2010), reinforcing the likelihood of phage involvement. An alternative explanation for LPS production in PTB could be that LPS production is stimulated during the bacterial stress response due to its role as a barrier molecule (Poole, 2012). A potential next step would be to sequence the bacteria grown between the two zones of clearing using both whole genome sequencing and RNA-Seq to examine any mutations or differences in gene expression from the freezer stock. Another possibility would be to directly locate the phage genes in *Dickeya* and track expression of specific known phage genes such as that of the transducing phage φEC2 (Adriaenssens et al., 2012). Overall, more work is necessary to examine PTB as a potential chemical treatment for bacterial infection of crops. Understanding *Dickeya*'s response to PTB would clarify the double-ring phenotype and help evaluate efficacy of PTB treatment and the ideal conditions under which treatment should be applied. Although present findings showed that all four *Dickeya* remained sensitive to PTB after treatment (Figure 2), further experiments should investigate the possibility of developing bacterial resistance after continued exposure to PTB to ensure its success as a plant treatment option.

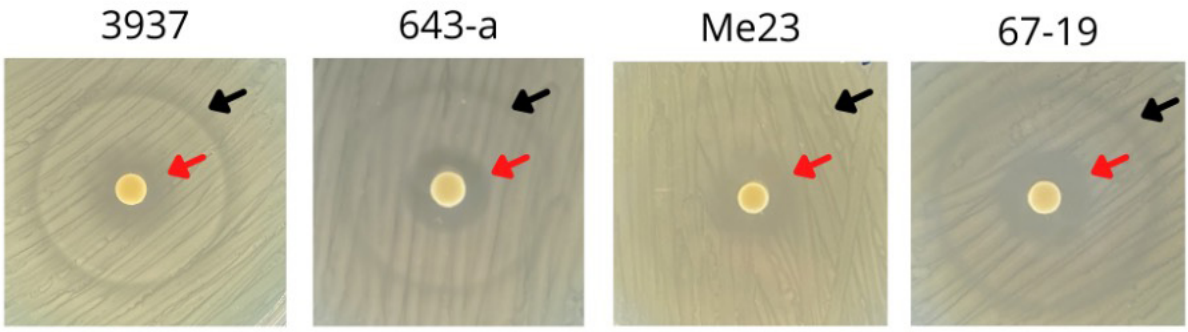

Figure 1: Disk diffusion assay of antibacterial activity of PTB on four strains of *Dickeya* freezer stocks:, *D. dadantii* 3937, *D. dianthicola* ME23, *D. dianthicola* 67-19, and *D. fangzhongdai* 643-a.

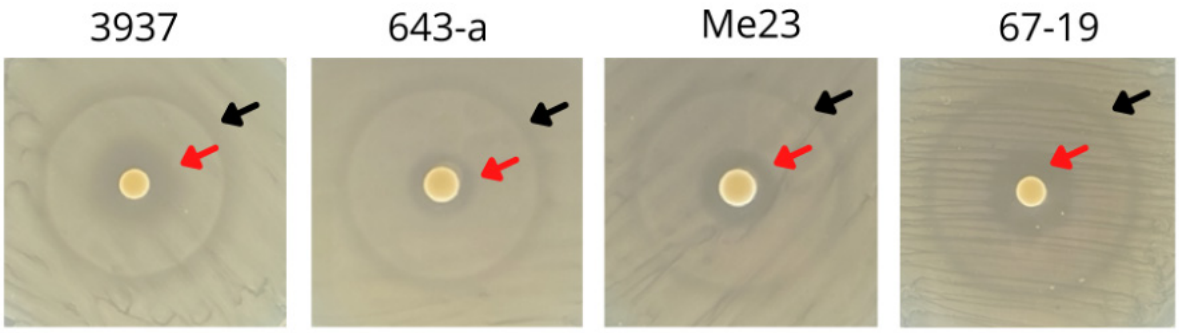

Figure 2: Disk diffusion assay of antibacterial activity of PTB on four strains of *Dickeya* re-streaked from the middle zone of growth between the two zones of clearing in Figure 1.The black arrows point to the outer zone of clearing and the red arrows to the inner zone.

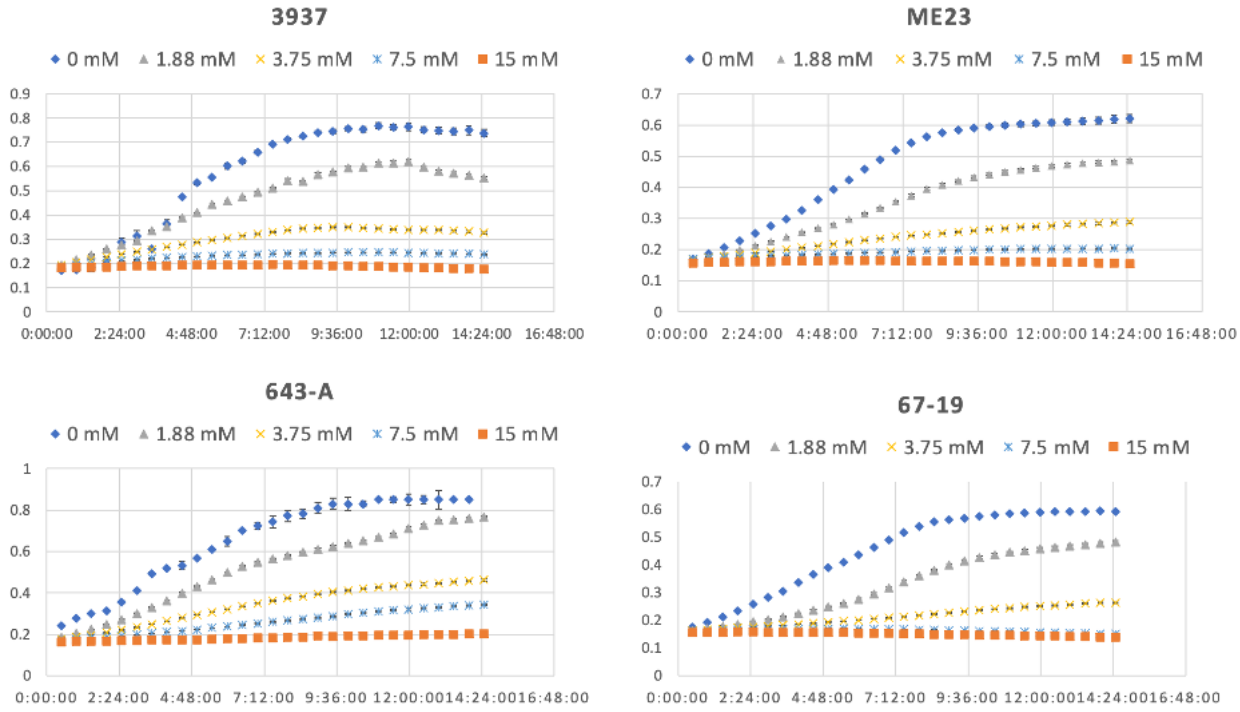

Figure 3: The growth (OD600) of four strains of *Dickeya*: *D. dadantii* 3937, *D. dianthicola* ME23, *D. dianthicola* 67-19 and *D. fangzhongdai* 643-a was measured for up to 16 hours. Each treatment had six replicates and each experiment was repeated at least three times. The most recent (4/30/2021)

Table 1. Genes found in the three *Dickeya* strains constructed for Tn-Seq libraries

Dikeya **dadantii 3937 Tn-Seq Genes**		
Genes that improved growth in PTB when disrupted	**Locus ID**	**Description**
	DDA3937_RS03410	polysaccharide biosynthesis protein
	DDA3937_RS03420	sugar transferase
	DDA3937_RS1865	mannose-1 phosphate guanylyltransferase/mannose 6-phosphate isomerase
	DDA3937_RS18660	phosphomannomutase / phosphoglucomutase
	DDA3937_RS18665	GDP-mannose 4,6-dehydratase
	DDA3937_RS18670	ABC transporter permease
	DDA3937_RS18685	WbeA
	DDA3937_RS18690	Glycosyltransferase
	DDA3937_RS20290	transcription/translation regulatory transformer protein RfaH
Genes that were detrimental to growth in PTB when disrupted	**Locus Id**	**Description**
	DDA3937_RS00900	cell division protein ZapB
	DDA3937_RS01600	outer membrane-stress sensor serine endopeptidase DegS
	DDA3937_RS02810	DedA family protein
	DDA3937_RS19930	uroporphyrinogen-III c-methyltransferase
	DDA3937_RS12215	sodium-potassium/proton antiporter ChaA
	DDA3937_RS05150	sigma E protease regulator RseP
	DDA3937_RS05640	ATP-dependent Clp endopeptidase proteolytic subunit ClpP
	DDA3937_RS05645	ATP-dependent protease ATP-binding subunit ClpX
	DDA3937_RS15765	RNA polymerase sigma factor RpoE
	DDA3937_RS06350	replication initiation negative regulator SeqA
	DDA3937_RS11035	redox-regulated ATPase YchF
	DDA3937_RS11210	septum site-determining protein minD
	DDA3937_RS18610	RNA chaperone Hfq
	DDA3937_RS12490	exoribonuclease II
Dickeya **dianthicola ME23 Tn-Seq Genes**		
Genes that improved growth in PTB when disrupted	**Locus Id**	**Description**
	DZA65_RS19720	mannose-1 phosphate guanylyltransferase / mannose 6-phosphate isomerase
	DZA65_RS19725	phosphomannomutase / phosphoglucomutase
	DZA65_RS19730	GDP-mannose 4,6-dehydratase
	DZA65_RS19755	glycosyltransferase
	DZA65_RS03635	sugar transferase
	DZA65_RS19750	hypothetical protein

Genes that were detrimental to growth in PTB when disrupted	DZA65_RS20165	microconductance mechanosensitive channel MscM
	DZA65_RS20220	glucose-6-phosphate isomerase
	DZA65_RS1610	envelope stress response regulator transcription factor CpxR
	DZA65_RS22635	4-amino-4-deoxy-L-arabinose-phosphoundecaprenol flippase subunit ArnE
	DZA65_RS22640	4-amino-4-deoxy-L-arabinose-phospho-UDP flippase
	DZA65_RS00800	O-antigen ligase family protein
	DZA65_RS01525	LPS export ABC transporter periplasmic protein LptC
	DZA65_RS09455	DUF3413 domain-containing protein
	DZA65_RS04310	Dyp-type peroxidase
Dickeya **dianthicola 67-19 Tn-Seq Genes**		
Genes that improved growth in PTB when disrupted	**Locus Id**	**Description**
	HGI48_RS18585	mannose-1 phosphate guanylyltransferase / mannose 6-phosphate isomerase
	HGI48_RS18590	phosphomannomutase CpsG
	HGI48_RS18595	GDP-mannose 4,6-dehydratase
	HGI48_RS06765	dTDP-4-dehydrorhamnose 3,5-epimerase
	HGI48_RS03520	sugar transferase
Genes that were detrimental to growth in PTB when disrupted	**Locus Id**	**Description**
	HGI48_RS19050	microconductance mechanosensitive channel MscM
	HGI48_RS20595	envelope stress response regulator transcription factor CpxR
	HGI48_RS20045	tyrosine recombinase XerC
	HGI48_RS05730	SmdA family multidrug ABC transporter permease/ ATP-binding protein
	HGI48_RS01595	DnaA initiator-associating protein DiaA

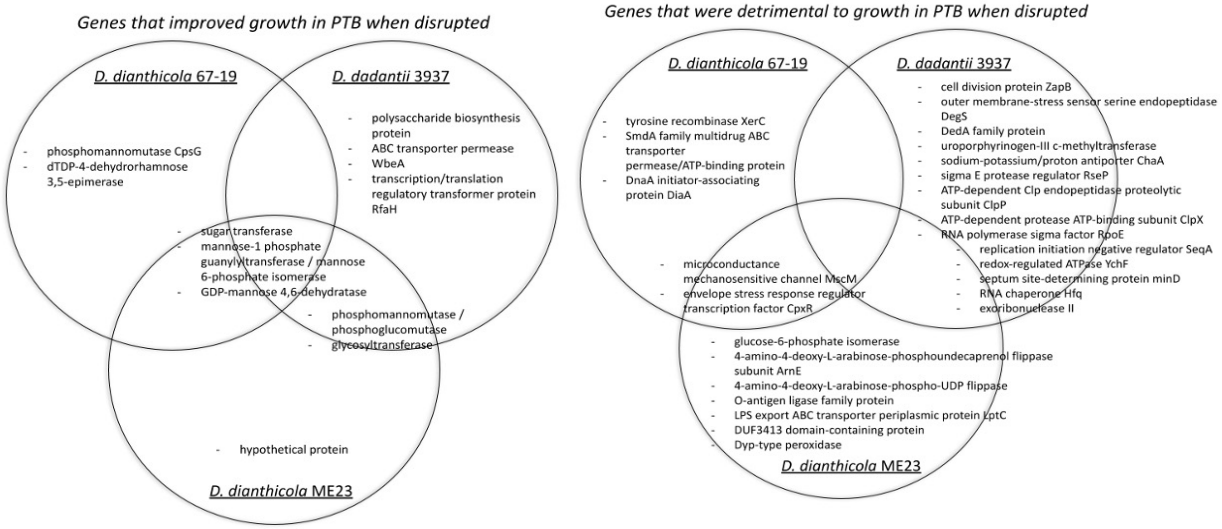

Figure 4: Venn diagrams of genes identified from Tn-Seq experiments. All experiments passed QC. Genes shown are all genes where |fit| > 2 and |t| > 4 and are sorted by fit value into genes that improved growth in PTB when disrupted (top) and genes that were detrimental to growth in PTB when disrupted

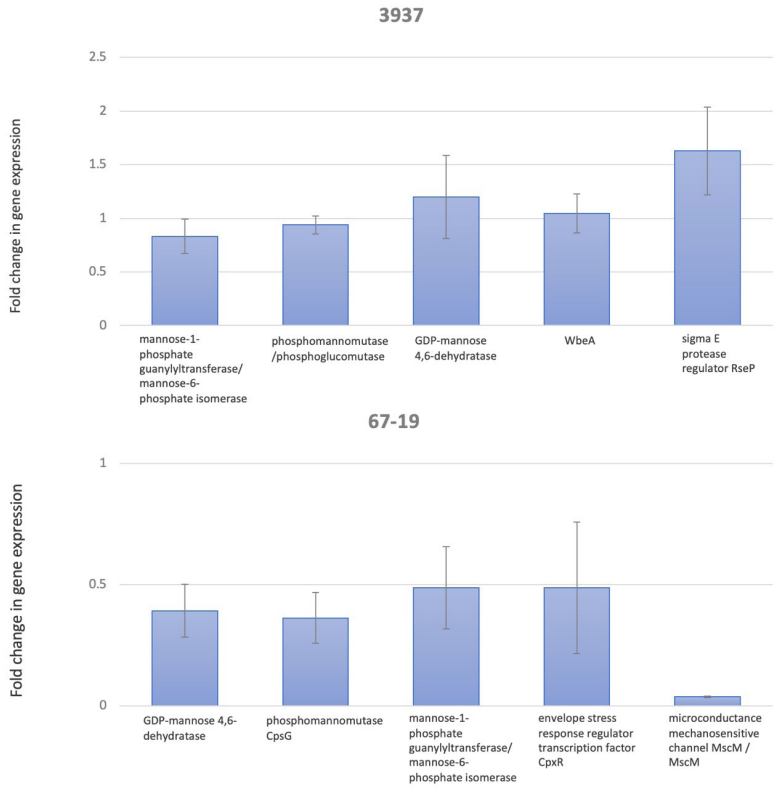

Figure 5: qRT-PCR fold change in gene expression for *D. dadantii* 3937 and *D. dianthicola* 67-19. The fold change in gene expression was calculated as a ratio of the gene expression of PTB-treated samples over the gene expression of non-treated samples. A fold change in gene expression < 1 would indicate that PTB-treated samples decreased expression of the gene of interest whereas a fold change in gene expression > 1 would indicate that PTB-treated samples increased expression of the gene of interest. Error bars indicate standard deviation of the mean.

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