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Regulation of D-galacturonate metabolism in *Caulobacter crescentus* by HumR, a LacI-family transcriptional repressor

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ABSTRACT

The oligotrophic freshwater bacterium *Caulobacter crescentus* encodes a cluster of genes (CC_1487 to CC_1495) shown here to be necessary for metabolism of D-galacturonate, the primary constituent of pectin, a major plant polymer. Sequence analysis suggests that these genes encode a version of the bacterial hexuronate isomerase pathway. A conserved 14 bp sequence motif is associated with promoter regions of three operons within this cluster, and is conserved in homologous gene clusters in related alpha-Proteobacteria. Embedded in the hexuronate gene cluster is a gene (CC_1489) encoding a member of the LacI family of bacterial transcription factors. This gene product, designated here as HumR (hexuronate metabolism regulator), represses expression of the *uxaA* and *uxaC* operon promoters by binding to the conserved operator sequence. Repression is relieved in the presence of galacturonate or, to a lesser extent, by glucuronate. Other genes potentially involved in pectin degradation and hexuronate transport are also under the control of HumR. Adoption of a LacI-type repressor to control hexuronate metabolism parallels the regulation of xylose, glucose, and maltose utilization in *C. crescentus*, but is distinct from the use of GntR-type repressors to control pectin and hexuronate utilization in gamma-Proteobacteria such as *Escherichia coli*.

Keywords: *Caulobacter*; Galacturonate; LacI

1. INTRODUCTION

Pectin is an important plant polysaccharide that aids the cross-linking of cellulose and hemicellulose to maintain

the integrity of plant cells and tissues [1]. The core pectin polymer consists of alpha-1, 4-linked D-galacturonate subunits, which are then modified by methylation and various oligosaccharide side chains depending on the plant source [1]. Metabolism of galacturonate, which makes up 40% - 70% of the dry weight of pectin, has been studied extensively in just a few microbes [2,3]. In this work, we show that the oligotrophic, stalked alpha-Proteobacterium *Caulobacter crescentus* degrades galacturonate through the hexuronate isomerase pathway, and regulates galacturonate metabolism via LacI-family repressor. This system for galacturonate metabolism and regulation is conserved in several closely related alpha-Proteobacteria, although the metabolic pathway had previously been observed only in gamma-Proteobacteria such as *Escherichia coli* and *Erwinia chrysanthemi*.

Microorganisms degrade pectin through a suite of secreted enzymes, with pectatelyase and exo-polygalacturonidase primarily responsible for chain depolymerization [2,4]. The most extensively studied pectinolytic bacterium is the phytopathogen *Erwinia chrysanthemi*, which attacks pectin to facilitate invasion of plant tissue during infection [5]. *Erwinia* extracts nutrients from pectin degradation products by consuming oligo-galacturonides, galacturonate, and 5-keto-4-deoxyuronate through the hexuronate isomerase pathway it shares with non-phytopathogenic Enterobacteriaceae such as *E. coli* [3].

Caulobacters are dimorphic alpha-Proteobacteria that often exhibit an adhesive stalk and readily form biofilms [6]. It is likely that species such as *C. crescentus* colonize decaying vegetation in aquatic habitats, secreting hydrolytic enzymes to access nutrients embedded in the insoluble cell walls [7]. Whole genome sequencing of *C. crescentus* revealed numerous genes encoding secretable enzymes for attacking cellulose, xylans, pectin, and other polymers [8]. Genomic analysis of closely related stalked

bacteria such as *Caulobacter henricii* strain K31 [9] and *Phenylobacterium zucineum* [10] shows comparable rosters of secretable hydrolases. In *C. crescentus*, there is evidence for coordinated expression of gene sets that include extracellular enzymes, transport systems, and cytoplasmic catabolic pathways when cells are exposed to relevant sugars, such as glucose and xylose [11]. For example, transcriptome analysis of *C. crescentus* metabolizing xylose revealed induction of xylanases and arabinosidases, putative outer and inner-membrane transporters, and a pathway for oxidative metabolism of xylose [11,12]. The xylose regulon is under the control of XylR, a member of the LacI family of bacterial transcription factors [13]. Our results reveal an analogous galacturonate regulon in *C. crescentus* that is under the control of another LacI family member, HumR. Given that the xylose operon promoter [14], in conjunction with XylR [13], has proven to be a robust tool for tightly controlled inducible gene expression in *C. crescentus*, the identification of HumR not only reveals commonalities in regulatory strategies, but may also expand the molecular genetic arsenal available for research applications in this organism.

2. MATERIALS AND METHODS

2.1. Bacterial Strains and Growth Conditions

Escherichia coli strains¹ were grown in Luria-Bertani (LB) broth or agar, supplemented as necessary with kanamycin (50 µg/ml), or oxytetracycline (10 µg/ml). *Caulobacter crescentus* strains were routinely grown in PYE broth or agar [15], supplemented as necessary with kanamycin (20 µg/ml in agar, 5 µg/ml in broth), oxytetracycline (2 µg/ml in agar, 1 µg/ml in broth), and/or nalidixic acid (20 µg/ml in agar). When indicated, cultures were grown on M2 minimal salts medium [15] containing the carbon/energy source at a final concentration of 10 mM. The D-isomer was used for all monosaccharides (galacturonate, glucuronate, glucose, galactose, and xylose). Two sources of pectin were tested: pectin derived from citrus fruits (P9135, Sigma-Aldrich Chemical Co.), and pectin derived from apple (P8471, Sigma-Aldrich Chemical Co.). A final concentration of 1.94 g/l pectin was used in media. Growth curves were conducted in 125 ml baffled flasks containing 50 ml PYE or M2 media. Cultures were incubated at 30°C in a bench top shaker at 250 - 300 rpm, and samples were taken periodically to monitor turbidity. Phenotype Microarrays (Biolog, Hayward, CA) [16] were also used to examine substrate utilization, as described previously [12]. The PM1 and PM2 plates contain 190 distinct carbon sources, and increases in culture turbidity were used to monitor productive substrate utilization.

¹Detailed descriptions of bacterial strains, plasmid constructs, and primers are available from the authors upon request.

2.2. Construction of Mutations

Knockouts were made by either integrative disruption or non-polar in-frame deletion [17]. Polymerase chain reaction (PCR) primers were synthesized by Integrated DNA Technologies (IDT; San Diego, CA). PCR was done using GoTaq thermostable polymerase (Promega Corp., Madison, WI). Chromosomal DNA was prepared using the DNEasy kit (Qiagen), according to the manufacturer's instructions. Integrative disruptions were constructed as described previously [17], resulting in insertion of the kanamycin-resistant pBGS18T plasmid into the target gene. Construction of non-polar in-frame deletions was done by a PCR-mediated process [17], which results in a deletion of coding sequence and no insertion. For integrative disruptions, the presence of integrated plasmid DNA was verified by PCR. Stability of the integrated plasmids was verified by growth under non-selective conditions (PYE), followed by plating in the absence or presence of kanamycin. After growth under non-selective conditions (PYE) for at least 10 generations, less than 1% of the population had lost the integrated plasmid-encoded kanamycin resistance. Deletion constructs were also verified by PCR.

2.3. Electrophoretic Mobility Shift Assays

C. crescentus strains were grown in 500 ml PYE cultures at 30°C to mid-log phase ($OD_{600} \sim 0.4$), and cell lysates were prepared as described previously [13]. Target DNA for binding assays was designed based on the 14 bp HumR operator sequence in the *uxaC* promoter region. The sequence was slightly modified outside of the putative operator motif to reduce predicted secondary structure, based on mFold [18]. Two 34-base oligonucleotides were synthesized: 5'-CTACGCGCGATGACACCGG-TTACCAGCAGACACG and 5'-CGTGTCTGCTGGT-AACCGGTGTCATCGCGCGTAG (putative HumR operator underlined). The 5' end of each was labeled with Cy5 (IDT). Oligonucleotides were annealed in equimolar amounts by first denaturing at 95°C for 5 min, then cooling slowly for 60 minutes to room temperature. Binding reactions consisted of 2.5 ng annealed duplex target DNA, binding buffer (150 mM KCl, 0.1 mM dithiothreitol, 0.1 mM EDTA and 10 mM Tris (pH 7.4)), 1 µg poly-dI: dC, and 22.5 µg protein in a total reaction volume of 15 µl. For binding inhibition assays, galacturonate, glucuronate, glucose, xylose, or galactose were added to 10 mM final concentration as appropriate. Reactions were incubated at room temperature for 10 minutes, and then resolved on a 6% non-denaturing polyacrylamide gel made and run with 1× Tris-borate-EDTA buffer. Gels were run at 100 V for 50 min, visualized on a Typhoon imaging system (Molecular Dynamics) and quantified using Image Quant software. Background counts were deter-

mined in lanes where no protein was added, and subtracted from the counts for lanes that contained cell extracts.

2.4. Expression Assays and Operator Mutants

To examine regulation of gene expression in response to galacturonate, the regions upstream of the CC_1488 (*uxaA*) and CC_1490 (*uxaC*) genes, respectively, were amplified by PCR. For wild-type promoters, PCR primers were designed to amplify up to 250 bp upstream of the start codons of these genes, along with a few hundred base pairs within the coding region. Synthetic EcoRI or HindIII sites were added to the 5' end of the primers to allow for directional cloning. PCR products were initially cloned into plasmid PCR2.1 using the TOPO Cloning System (Life Technologies), then subcloned into pRKlac290, a low copy-number, broad host range plasmid to generate transcriptional fusions to the *lacZ* gene [19]. pRKlac290 constructs were transferred from *E. coli* S17.1 by conjugation into *C. crescentus* strains, with selection on PYE supplemented with nalidixic acid and oxytetracycline. Construction of targeted mutations in the putative operator for the *uxaC* promoter was done with a two-step PCR-based method [17]. Promoter constructs containing the mutant operators were cloned as described above, and β -galactosidase activity was assayed as described previously [13,19].

2.5. qRT-PCR Analysis of Gene Expression

Quantitative real-time PCR (qRT-PCR) was used to examine gene expression, starting with RNA isolated from *C. crescentus* strains grown in PYE medium, with or without galacturonate. RNA was extracted as described previously [11]. Isolated RNA was subjected to two consecutive genomic DNA removal treatments by digesting with 2 U DNaseI (Ambion) per 10 μ g RNA, incubated at 37°C for 60 min. DNaseI was inactivated by addition of phenol:chloroform (5:1) and RNA was precipitated with 3M sodium acetate (pH 5.2) and 100% ethanol at -20°C overnight. Pellets were obtained by centrifugation at 16,000 \times g for 15 min, washed with 70% ethanol and re-suspended in RNase free water. RNA purity was determined spectrophotometrically and agarose gels were run to verify RNA integrity.

Complementary DNA (cDNA) was synthesized with 2.0 - 2.5 μ g of RNA and SuperScriptII Reverse Transcriptase (200 units, Life Technologies) with random primers (100 ng per 5 μ g total RNA, Life Technologies) following the manufacturer's suggested protocol, including the optional RNaseH digestion (New England Biolabs). Primer and probe sequences for qRT-PCR were designed using the PrimerExpress v3.0 software (Life Technologies) and synthesized by IDT. Because primers did not

include the minor groove binder, the probe T_m parameter was adjusted to be 10°C higher than the primer T_m values. Analyses for heterodimer and homodimer formation and secondary structure were done using IDT's OligoAnalyzer 3.1, and BLAST was used to examine the potential for non-target site primer/probe annealing. Specificity of primers and probes was subsequently verified by analyzing qRT-PCR products on agarose gels. Probes were labeled with the 5' reporter dye 6-FAM and the 3' quencher Iowa Black FQ. qRT-PCR was performed on a StepOne Real Time PCR System (Life Technologies) in 20 μ l reaction volumes consisting of 1 \times TaqMan Gene Expression Master Mix, 900 nM forward and reverse primers, 250 nM probe, and 500 ng of cDNA. Thermocycling conditions were: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec. Amplification efficiency of each primer and probe set was determined using a standard curve. The five dilutions of the standard curve were tested in triplicate and were serially diluted five-fold from a starting quantity of 125 ng/ μ l. In each experiment, samples were run in duplicate and two internal controls were used to normalize results [20]. The genes used for normalization were *rpoA* (CC_1272), which encodes the RNA polymerase alpha subunit, and *secA* (CC_3068), which encodes the pre-protein translocase subunit SecA. These controls were chosen based on expected constitutive expression under all conditions used, and because their functions are unrelated to the genes under investigation. In addition, raw RNA extract and CB15 genomic DNA were included to detect the presence of contaminating genomic DNA.

Threshold levels for each gene were adjusted to control for inter-assay variation and the relative expression of each gene was determined with the comparative $\Delta\Delta C_T$ method [21]. The ΔC_T s for each sample and target were first obtained by normalizing the threshold cycle number [C_T] to the mean C_T of constitutively expressed *rpoA* and *secA* genes. Relative quantification (RQ) values were calculated using the equation $RQ = (1 + E)^{-\Delta\Delta C_T}$, where E is the amplification efficiency of each target determined by a standard curve and $\Delta\Delta C_T = \Delta C_{T\text{reference}} - \Delta C_{T\text{sample}}$ [22].

2.6. RT-PCR of Hexuronate Metabolism Genes

To determine whether closely spaced genes involved in galacturonate metabolism are encoded on the same transcript, reverse transcriptase-mediated PCR was employed for the following gene pairs: CC_1487-1488 (*uxaB* and *uxaA*), CC_1490-1491 (*uxaC* and *kduI*), and CC_1495-1496 (*kdgA* and *kdgK*). Primers were designed with the forward primer annealing 150 - 300 bp upstream of the 3' end of the first gene, and the reverse primer annealing in the coding region of the putative linked downstream gene. As a control, primers were designed for the

CC_3040-3041 gene pair (*fliI* and *fliJ*), which are known to be co-transcribed in a polycistronic mRNA. RNA samples were prepared as described above and used as a template for cDNA synthesis. CB15 genomic DNA was included as a positive control. Templates were diluted to 100 ng/ μ l and PCR was performed in 25 μ l reaction volumes containing 1 \times Green GoTaq Buffer (Promega), 0.25 mM dNTPs, 1 μ M each forward and reverse primer, 0.75 U GoTaq DNA Polymerase (Promega) and 1 μ l template. Thermocycling conditions were: 95°C for 2 min, followed by 30 cycles of 95°C for 15 sec, 60°C for 25 sec, and 72°C for 50 sec, with a final 5 min extension at 72°C. Products were visualized by agarose gel electrophoresis using 0.9% agarose, and the gels were subsequently stained with ethidium bromide.

3. RESULTS

3.1. Growth of *C. crescentus* on Hexuronates

Analysis of the *C. crescentus* CB15 genome sequence suggested that it encodes enzymes for the degradation of several major plant polysaccharides, including cellulose, xylan, lignin, glucan, and pectin [8]. To investigate pectin and hexuronate utilization, growth of *C. crescentus* was examined using pectin, galacturonate, and glucuronate as sole carbon sources. (The D-isomer of all monosaccharides was used in this work.) Pectin produced mixed results. Under conditions in which glucose produced robust colonies in 3 days, pectin derived from apple peel yielded small colonies in 6 days, and pectin derived from citrus fruit did not support visible growth. Pectin from different plant sources can differ significantly in structure and digestibility [1], but the results with apple peel pectin show that *C. crescentus* is capable of consuming at least some forms of this polymer. The core pectin chain is comprised of alpha-1,4-linked galacturonate. Pure galacturonate (10 mM) supported growth of *C. crescentus* on plates, with small colonies visible after 4 - 5 days. In liquid M2 medium, the log phase generation time on galacturonate was approximately 4.3 hours, roughly twice as long as doubling times on glucose (2.0 h) or xylose (2.3 h). Adaptation to growth on galacturonate was slow; wild-type cells transitioning from glucose to galacturonate in M2 medium experienced a prolonged lag phase of 24 h or more before initiating exponential growth, roughly three-fold longer than the lag phase in transitioning from glucose to xylose. Growth on glucuronate was comparable to growth on apple pectin, suggesting that *C. crescentus* "prefers" galacturonate over glucuronate.

3.2. Genetic Characterization of Galacturonate Utilization

In bacteria, pectate (demethylated pectin) can be cleaved by pectatelyase and/or exo-poly-galacturonidase to gen-

erate oligo-galacturonides and galacturonate for import to the cytoplasm [2]. Two bacterial pathways have been identified for galacturonate metabolism [3]: the hexuronate isomerase pathway studied extensively in *E. coli* and *E. chrysanthemi*, and the oxidative pathway active in *Agrobacterium tumifaciens* and certain *Pseudomonas* species. Although *C. crescentus* is much more closely related to *A. tumifaciens* (an alpha-Proteobacterium) than to *E. coli* or *E. chrysanthemi* (gamma-Proteobacteria) [23], the *C. crescentus* genome contains a set of genes encoding the gamma-Proteobacterial hexuronate isomerase pathway, and lacks a close relative of the galacturonate dehydrogenase enzyme that is key to the *A. tumifaciens* pathway [24]. The genomic region containing the genes of the putative *C. crescentus* hexuronate metabolism pathway is shown in **Figure 1**. This gene cluster is likely organized as several operons, based on orientation and gene spacing (**Figure 1(A)**). Reverse transcriptase-mediated PCR confirmed that CC_1488 and CC_1487 are transcribed on the same mRNA, as are CC_1490-1491-1492, and CC_1495-1496 (data not shown). **Figure 1(B)** shows the hexuronate isomerase pathway enabled by the products of this gene cluster [3,5]. The penultimate step generates 2-keto-3-deoxy-6-phospho-D-gluconate, an intermediate shared with the Entner-Doudoroff pathway, the sole route of glucose catabolism in *C. crescentus* [11,25]. The final step, catalyzed by keto-deoxyphosphogluconate aldolase (KdgA, CC_1495 gene product), yields pyruvate and glyceraldehyde-3-phosphate. We have shown previously that CC_1495 is necessary for glucose metabolism [11].

To determine whether genes in this cluster are necessary for growth on galacturonate, a series of mutant strains were constructed and tested for growth on M2 media using galacturonate as sole carbon and energy source (**Table 1**). As controls, the mutant strains were also tested for growth on glucose and xylose. Strains containing mutations in the CC_1487 (*uxaB*), CC_1488 (*uxaA*), CC_1490 (*uxaC*), and CC_1495 (*kdgA*) genes were incapable of growth on galacturonate. The *kdgA* disruption mutant, like the strains with point mutations in *kdgA* investigated previously, is also incapable of growth on glucose, as the encoded enzyme catalyzes an essential step in the Entner-Doudoroff pathway. Mutations in genes encoding enzymes catalyzing "upstream" reactions in the Entner-Doudoroff pathway (CC_2054-2057) [11] blocked glucose utilization, but had no effect on galacturonate metabolism. The *uxaB*, *uxaA*, and *uxaC* mutants grow normally on glucose and xylose, and the only growth defects relative to wild-type *C. crescentus* apparent on Biolog Phenotype Microarrays were with galacturonate and glucuronate. (Pectin did not support significant growth of *C. crescentus* in this assay.) Strains containing mutations in CC_1491 (*kduI*) and CC_1492 (*kduD*) grew normally on galacturonate, consistent with their inferred role in the

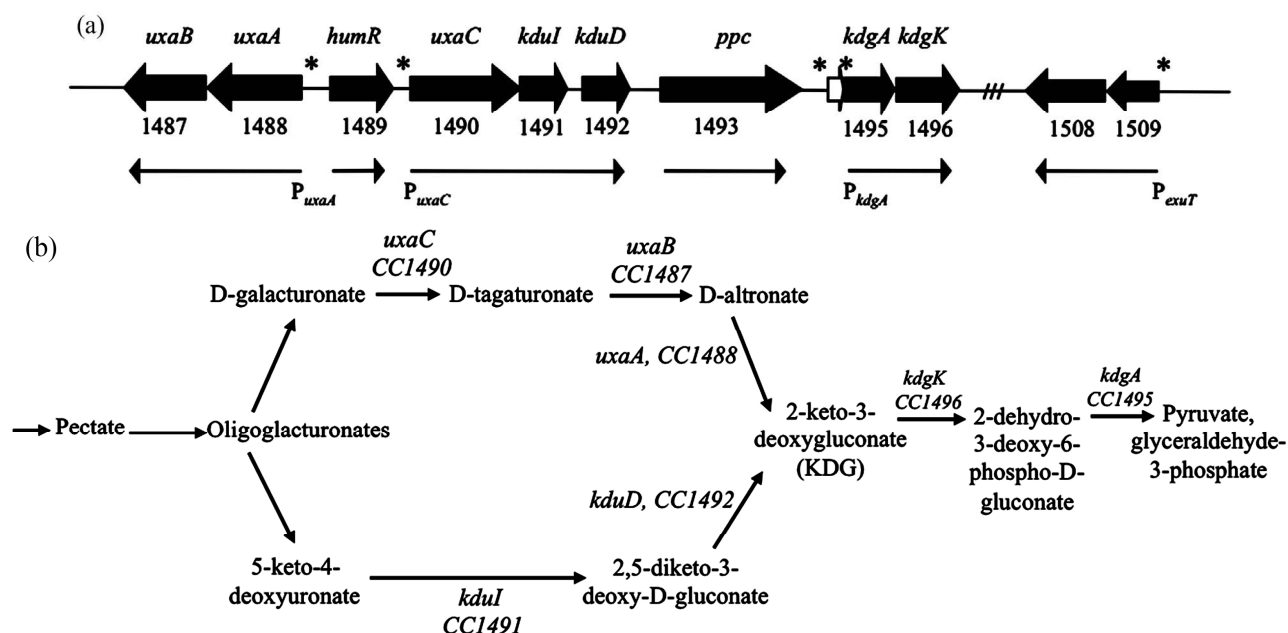


Figure 1. A putative pathway for D-galacturonate degradation in *C. crescentus*. A) The CC_1487–1509 genome region in *C. crescentus* CB15. The CC_1494 gene predicted in the original *C. crescentus* genome annotation is shown in white. The putative CC_1494 ORF (128 codons) has no significant relatives in GenBank. The CC_1497 to CC_1507 region is not shown [hash marks] as their predicted functions do not appear to be connected to hexuronate utilization; B) Proposed *C. crescentus* pectin/hexuronate degradation pathway. Polygalacturonides derived from pectin are degraded to yield monomeric galacturonate or 5-keto-4-deoxyuronate, which are processed through parallel pathways merging at 2-keto-3-deoxygluconate (KDG). The *C. crescentus* genes hypothesized to encode the enzyme responsible for each step in the pathway are indicated. Gene names are based on homology to genes of known function in *E. coli* and *E. chrysanthemi*.

Table 1. Growth of *C. crescentus* strains containing mutations in genes encoding putative enzymes of the galacturonate metabolic pathway. “–” indicates that no individual colonies formed on M2 minimal salts agar with the indicated sole carbon and energy source within 7 days. “±” indicates that colonies less than half the size of the wild-type control after 7 days. “+” indicates colonies that formed at a similar rate and size to wild-type.

Disrupted gene	Annotated function	Growth with sole carbon/energy source:		
		Galacturonate	Glucose	Xylose
CC_1487 <i>uxaB</i>	D-tagaturonate reductase	–	+	+
CC_1488 <i>uxaA</i>	D-altronate dehydratase	–	+	+
CC_1489 <i>humR</i>	Transcriptional regulator, LacI-GalR family	+	+	+
CC_1490 <i>uxaC</i>	Glucuronate isomerase	–	+	+
CC_1491 <i>kduI</i>	5-keto-4-deoxyuronate isomerase	+	+	+
CC_1492 <i>kduD</i>	2-deoxy-D-gluconate 3-dehydrogenase	+	+	+
CC_1493 <i>ppc</i>	Phosphoenolpyruvate carboxylase	–	–	+
CC_1495 <i>kdgA</i>	2-keto-3-deoxyphosphogluconate aldolase	–	–	+
CC_1496 <i>kdgK</i>	2-dehydro-3-deoxyglucokinase	±	±	+

parallel branch that processes 5-keto-4-deoxyuronate, a product of pectate lyase.

The CC_1496 mutant strain grew slower on galacturonate relative to wild-type, but still formed detectable colonies, indicating that this gene product is important but not essential for galacturonate metabolism. We des-

ignated CC_1496 as *kdgK*, based on its genome location and weak sequence similarity to KdgK (2-keto-3-deoxygluconate kinase) gene products, but the knockout results suggest that it may not be the only gene product able to carry out this phosphorylation reaction.

The *ppc* gene (CC_1493) is necessary for growth of *C.*

crenscentus on glucose and galactose [11], and was found here to be essential for growth on galacturonate. CC_1493 encodes phosphoenolpyruvate (PEP) carboxylase, which catalyzes the addition of CO₂ to PEP to generate oxaloacetate. During growth on minimal media with glucose or galacturonate as the sole carbon input, PEP carboxylase may serve an anaplerotic function, replenishing oxaloacetate levels to allow the TCA cycle to continue as intermediates are withdrawn for use in other biosynthetic pathways.

3.3. Regulation of Galacturonate Metabolism

To determine whether expression of genes implicated in galacturonate metabolism is induced in response to galacturonate in the culture medium, the regions upstream of *uxaA* (CC_1488) and *uxaC* (CC_1490) were inserted next to a promoterless *lacZ* reporter on a low copy-number plasmid vector. β -galactosidase activity was assayed in wild-type *C. crescentus* grown in PYE medium [15] before and after exposure to 10 mM galacturonate, glucuronate, or other sugars. In plain PYE, promoter activity was very low in both constructs ($P_{uxaA} = 14 \pm 24$ Miller units, $P_{uxaC} = 54 \pm 29$). In the presence of galacturonate, promoter activity increased dramatically ($P_{uxaA} = 239 \pm 139$, 17-fold increase; $P_{uxaC} = 1150 \pm 214$, 21-fold increase). Subsequent analysis by quantitative real-time PCR (qRT-PCR) showed that both promoters were fully induced within 5 minutes of exposure to galacturonate. P_{uxaA} was relatively weak compared to P_{uxaC} , but the behavior of the two promoters was qualitatively similar. Glucuronate also induced expression of both promoters, though somewhat less effectively than galacturonate ($P_{uxaA} = 15$ -fold increase; $P_{uxaC} = 5$ -fold increase). Neither glucose, galactose, or xylose induced either promoter, nor did they prevent induction of these promoters by galacturonate. Expression of these promoters was also examined in cultures grown in M2 medium, with similar results (data not shown).

The CC_1489 gene, located in the midst of the hexuronate metabolism gene cluster, encodes a member of the LacI repressor family. The polypeptide sequence encoded by CC_1489 is similar to *C. crescentus* XylR (CC_3065; 38% identity over 438 amino acids with CC_1489), which controls a gene set for xylose metabolism [11,13]. To determine whether CC_1489 controls genes involved in galacturonate metabolism, an integrative disruption was constructed. The disruption mutant exhibited a log phase growth rate virtually identical to wild-type on M2 minimal medium supplemented with 10 mM glucose, xylose, galacturonate, or glucuronate, but transitioned more quickly to logarithmic growth when transferred from glucose to galacturonate or glucuronate. When expression of the *uxaA* and *uxaC* promoters (P_{uxaA} and P_{uxaC} , respectively) was examined in the CC_1489 disruption mutant, both became highly active under non-inducing conditions, exceeding the activity of the promoters in wild-type CB15 in the presence of galacturonate (Table 2 line 1; P_{uxaA} results not shown). In the CC_1489 mutant background, addition of galacturonate did little to increase promoter activity beyond that in plain PYE. These observations are consistent with the CC_1489 product acting as a repressor of P_{uxaA} and P_{uxaC} in the absence of galacturonate. We therefore have designated the CC_1489 gene product HumR, for *hexuronate metabolism regulator*.

MEME [26] was used to search for conserved sequences in the intergenic regions of the CC_1487-1496 cluster, to identify candidate binding sites for potential regulatory factors. The annotated CC_1494 coding region was included in the search, since this small ORF is not conserved at the protein level in any other species and we suspect that it may not actually encode a polypeptide. A conserved 14 bp sequence (TGACACC|GGTTACC) was identified upstream of the *uxaA*, *uxaC*, and *kgdA* genes in the *C. crescentus* genome, and in similar locations upstream of these genes in *C. henricii* K31,

Table 2. Regulation of P_{uxaC} -*lacZ* expression by HumR and galacturonate. Promoter constructs with identical 5' and 3' ends and sequences, save for the mutations shown, were inserted upstream of a promoterless *lacZ* gene in the low copy number pRKLac290 reporter vector. The resulting β -galactosidase activities (Miller units) are shown in the table.

Promoter	Operator	CB15 (wild-type)			<i>humR</i> disruption		
		PYE	PYE + gal	Ratio (+/- gal)	PYE	PYE + gal	Ratio (+/- gal)
P_{uxaC}	TGACACCGTTACC	52 ± 28	1090 ± 165	21	3200 ± 54	4900 ± 498	1.5
Mut A	TGACAGAGGTTACC	3310 ± 208	3540 ± 374	1.1	3610 ± 132	3160 ± 177	0.9
Mut B	TGAGGCCGTTACC	4810 ± 891	4520 ± 578	0.9	4970 ± 191	4410 ± 112	0.9
Mut C	TGACACCTAGTACC	2810 ± 692	2580 ± 287	0.9	2500 ± 280	2250 ± 289	0.9
Mut D	TGACACCGTTTCA	43 ± 15	85 ± 4	2.0	– ¹	– ¹	– ¹

¹Promoter activity was not detectable, as measured β -galactosidase activity was at or below background levels for the parental strain. Because of this, some activity ratios could not be calculated for this construct.

Caulobacter segnis, *P. zucineum*, and *A. excentricus*. To determine whether this motif is relevant to regulation, four distinct multi-base mutations were generated in the motif upstream of *C. crescentus uxaC* to examine their effects on gene expression and regulation (Table 2). With three of the mutations (Tables 2(A)-(C)), P_{uxaC} was expressed at high levels in the absence of galacturonate, and was not further activated by genetic elimination of HumR. These results are consistent with an operator site responsible for interaction with a repressor. By contrast, “MutD”, a 2 bp change in the far right of the putative operator sequence, virtually eliminated P_{uxaC} expression under inducing conditions. A possible explanation for this is presented in the “Discussion” section.

To determine whether HumR binds to the conserved operator, an electrophoretic mobility shift assay was carried out using a fluorescently-labeled synthetic *uxaC* operator. Crude extracts from wild-type *C. crescentus* formed a distinct shifted complex with the synthetic operator (Figure 2, lane 2) that was virtually eliminated when extracts from the *humR* mutant were used (Figure 2, lane 3). In the presence of 10 mM galacturonate or glucuronate, the intensity of the wild-type complex was greatly reduced (Figure 2, lanes 4 and 5). Glucose and galactose had no effect on the complex, demonstrating specificity of HumR for hexuronates. These observations are consistent with a LacI-type model in which HumR loses affinity for the operator when inducer (galacturonate or glucuronate) is present, allowing transcription to proceed. A faint lower-molecular weight complex was also seen that was present for all strains tested, and with all sugars. This band may reflect another protein able to

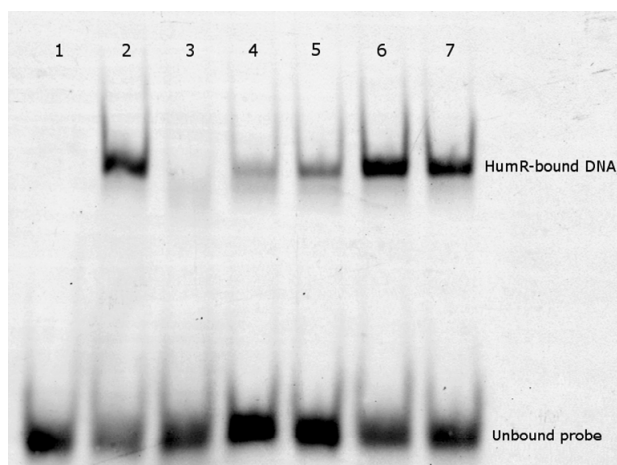


Figure 2. Gel mobility shift assay demonstrating HumR binding to a synthetic P_{uxaC} operator. Lane 1, no cell extract added; lane 2, CB15 cell extract; lane 3, *humR* mutant cell extract; lane 4, CB15 cell extract with 10 mM galacturonate; lane 5, CB15 cell extract with 10 mM glucuronate; lane 6, CB15 cell extract with 10 mM galactose; lane 7, CB15 cell extract with 10 mM glucose.

interact weakly with the synthetic probe.

3.4. The HumR Regulon

To identify other genes potentially regulated by HumR, the MEME-identified conserved motif was used to search the *C. crescentus* genome [8]. After filtering the results to focus only on sequences in intergenic regions, several potential operators were identified (Table 3). Within the CC_1487-1495 cluster, a second potential operator sequence upstream of CC_1494-1495 shared 11 out of 14 bp. Other potential operators included a 14/14 bp match located 116 bp upstream of the start codon for CC_1446, the first gene in a three gene unit (CC_1446–1448) annotated as encoding the three key components of an inner membrane TRAP family transport system that could potentially serve as an ATP-independent transporter for hexuronates [27]; a 13/14 bp match located 79 bp upstream of CC_1509, the first gene in a likely operon that includes a homolog of the *exuT* hexuronate transporter (CC_1508) [28]; an 11/14 bp match located 99 bp upstream of the start codon for CC_3152, which is annotated as encoding a pectatelyase; and an 11/14 bp match located upstream of CC_0442, a putative TonB-dependent outer membrane receptor.

To determine whether other potential HumR operators can convey HumR- and galacturonate-dependent regulation of transcription, expression of CC_1446, CC_1509, and CC_3152 was assayed by qRT-PCR. Because the gene expression analyses presented earlier were based on *lacZ* fusions, we also examined *uxaA* and *uxaC* expression by qRT-PCR. RNA was obtained from both wild type and *humR* mutant strains grown in PYE medium in the presence or absence of galacturonate. The cultures all had very similar growth rates and were harvested at similar densities. All of the genes with potential HumR operators showed induction by galacturonate, and were activated in the *humR* mutant strain in the absence of galacturonate (Table 4). It is not clear why the apparent magnitude of regulation for P_{uxaA} and P_{uxaC} varies substantially when assayed by different techniques. Effective qRT-PCR primer and probe sets could not be designed for CC0442 and CC1494, but microarray data (unpublished) has shown that expression of these mRNA's is strongly regulated by HumR and galacturonate, placing them in the HumR regulon as well.

4. DISCUSSION

4.1. Utilization of Pectin and Galacturonate

Caulobacter species are not known to act as plant pathogens, but likely scavenge decaying plant biomass in aquatic environments. The data presented here show that *C. crescentus* is capable of consuming at least some

Table 3. Possible HumR operator sequences upstream of genes potentially involved in galacturonate acquisition, transport, and degradation. The sequences shown are all in intergenic regions. See “Results” section for more on predicted functions of each gene.

Adjacent gene	Putative operator sequence	Orientation of operator relative to promoter	Distance from start of operator to translational start [bp]
CC_1488	TGA CAC CGG TTA CC	+	69
CC_1490	TGA CAC CGG TTA CC	+	75
CC_1495	TGA CAC CGG TTA CC	-	385
CC_1495	TGA CAC CGG TGG CA	+	435 ¹
CC_0442	TGA CAC CGG TGG CA	+	129
CC_1446	TGA CAC CGG TTA CC	+	115
CC_1509	TGA CAC CGG TTT CC	+	78
CC_3152	TGA CAC CGG TGGAC	+	96

¹Distance to the annotated CC_1494 translational start is 44 bp. As described in the legend to **Figure 1**, the legitimacy of CC-1494 as a functional coding region is uncertain.

Table 4. qRT-PCR analysis of gene expression and regulation by HumR and galacturonate. Strains used were wild-type CB15, and a *humR* disruption mutant in the CB15 background. Cultures were grown in PYE medium, and RNA was isolated from cultures at OD_{600nm} ~ 0.3. “Induced” cultures had galacturonate added to 10 mM final concentration 1 hour before the culture reached the target density. See “Results” section for more on predicted functions of each gene.

Gene	<i>humR</i> /WT ratio	Induced/uninduced ratio
CC_1488	53.2 ± 19.8	7.00 ± 2.85
CC_1490	50.0 ± 25.9	6.51 ± 2.79
CC_1446	2.48 ± 0.51	2.01 ± 0.41
CC_1509	111 ± 30	73.8 ± 20.5
CC_3152	14.1 ± 2.2	9.14 ± 1.46

forms of pectin, and grows well on galacturonate, pectin’s core monosaccharide. Genetic evidence indicates that *C. crescentus* metabolizes galacturonate via a pathway starting with hexuronate isomerase, while a parallel pathway processes 5-keto-4-deoxyuronate. The two branches start with distinct pectin breakdown products, probably to accommodate the use of both pectate lyase and polygalacturonidase. Analysis of the *C. crescentus* genome shows two putative pectate lyases (CC_3152 and CC_2035) and a putative exo-poly-alpha-galacturonosidase (“exo-PG”, CC_0572), each predicted by SignalP to contain an N-terminal signal peptide whose presence is consistent with secretion. In *E. chrysanthemi*, exo-PG and pectate-lyase are coordinately regulated, and are both needed for maximally efficient breakdown of pectin [29]. Whether this is true in *C. crescentus* requires further investigation.

The optimization of *C. crescentus* for utilization of galacturonate, rather than glucuronate, would be sensible if galacturonate, as a component of pectin, is more abundant

than glucuronate in niches this freshwater microbe is likely to inhabit. The underlying physiological explanation for this adaptation would require comprehensive analysis of transport, regulation, and enzymatic activities. Since glucuronate is capable of inducing the HumR regulon, it presumably is imported into the cell, suggesting that the reason for inefficient growth is due to differential metabolism in the cytoplasm.

4.2. Regulation by HumR

Expression of several genes implicated in galacturonate utilization in *C. crescentus* is controlled by HumR, a member of the LacI family of bacterial transcription factors [30]. There are 11 LacI family members encoded in the *C. crescentus* genome. Other are known to control uptake and/or metabolism of xylose (CC_3065), glucose (CC_2053), and maltose (CC_2284) [11,13,21]. Data presented here indicates that HumR binds to cognate operators in the absence of galacturonate, and interferes with effective RNA polymerase interaction with the respective promoter. The data are consistent with galacturonate binding to HumR, stabilizing a conformation with reduced affinity for operator DNA and releasing it from the promoter region to allow gene expression. Glucuronate also appears to productively interact with HumR as well, both *in vitro* and *in vivo*.

HumR functions similarly to the *C. crescentus* xylose repressor, XylR [13]. The XylR regulon includes a xylose transporter and an operon encoding enzymes for xylose metabolism, as well as putative secretive xylanases and arabinosidases [11]. The HumR operator (TGACACC|GGTTACC) and XylR operator (TGTTAGC|GCTACCA) sequences are the same length (14 bp), and identical at 7 out of 14 positions [13]. Given that LacI-type repressors bind as dimers, dyad symmetry is expected in the operator. All of the identified HumR operators are identical in

one half-site (TGACACC), which we suspect may be the optimal binding sequence for a HumR subunit. We have been unable to experimentally determine the 5' end of a HumR-regulated transcript, but alignment of P_{uxaC} sequences from the *Caulobacter* group (**Figure 3(a)**) reveals additional conserved sequences flanking the operator that match the -10 and -35 elements of P_{xyl} (**Figure 3(b)**), for which the 5' end is known. In P_{xyl} , the XylR operator is positioned between the -35 and -10 regions, overlapping the predicted downstream contact site for the σ subunit of RNA polymerase. If the aligned sequences represent the same functional promoter elements in P_{xyl} and P_{uxaC} , then bound HumR would likewise overlap the -10 region, allowing it to block critical contacts with the σ subunit. If that is true, the sequence of the right half-site of the HumR operator in P_{uxaC} is probably under dual selection for binding HumR and σ . This is consistent with the effect of OpMutD, a 2 bp change on the far right side of the operator (**Table 2**) that actually makes the sequence of the HumR operator half site more similar to the left side consensus, but changes the putative -10 region away from that shared with P_{xyl} . **Table 2** shows that promoter activity was dramatically reduced by this mutation, even under conditions where HumR binding should be eliminated, supporting the hypothesis that the operator overlaps with a critical promoter element.

As with the XylR regulon, some HumR-regulated promoters show mild reductions in inducibility when glucose is present, but glucose does not preclude induction of XylR or HumR-regulated promoters. There are no significantly conserved sequence elements in these promoters other than the identified operators, so direct involvement of additional glucose-responsive transcription factors

such as CAP (*E. coli*) or CcpA (*Bacillus subtilis kduID* operon [31]) seems unlikely. As an oligotroph adapted to low-nutrient habitats, there may be minimal reward for *C. crescentus* to prioritize glucose utilization at the expense of other available sugars.

The HumR regulon in *C. crescentus* includes at least five inferred operons (CC_1488-1487, CC_1490-1491-1492, CC_1495-1496, CC_1509-1508 and CC_1446-1448) and two singleton genes (CC_3152 and CC_0442). Based on annotated functions, the components of the HumR/galacturonate regulon fit the pattern previously established for the XylR/xylose regulon [11,13], and for glucose-dependent gene expression [11], in that they all include extracellular polysaccharide-active enzymes, TonB-dependent outer membrane receptors (TBDR's), inner membrane transport systems, and a cytoplasmic catabolic pathway. Thus, each repressor likely controls a complete system for extracellular polysaccharide degradation, uptake of breakdown products, and intracellular catabolism of monosaccharides to yield energy and central metabolites. The use of TBDR's for scavenging polysaccharide degradation products is shared by phytopathogenic *Xanthomonas* species, and Blanvillain *et al.* [32] recognized that TBDR's tend to be genetically clustered with "carbohydrate active enzymes" and inner membrane transport systems to create "CUT" (carbohydrate-utilization) loci. *Caulobacter crescentus* apparently did not maintain tight genetic linkage of CUT systems over evolutionary time, but still coordinates expression of components through repressors that recognize specific monosaccharides as induction signals.

In *E. coli* and *Erwinia chrysanthemi*, pectin degradation and hexuronate utilization are primarily controlled by the ExuR, UxuR, and KdgR transcription factors spe-

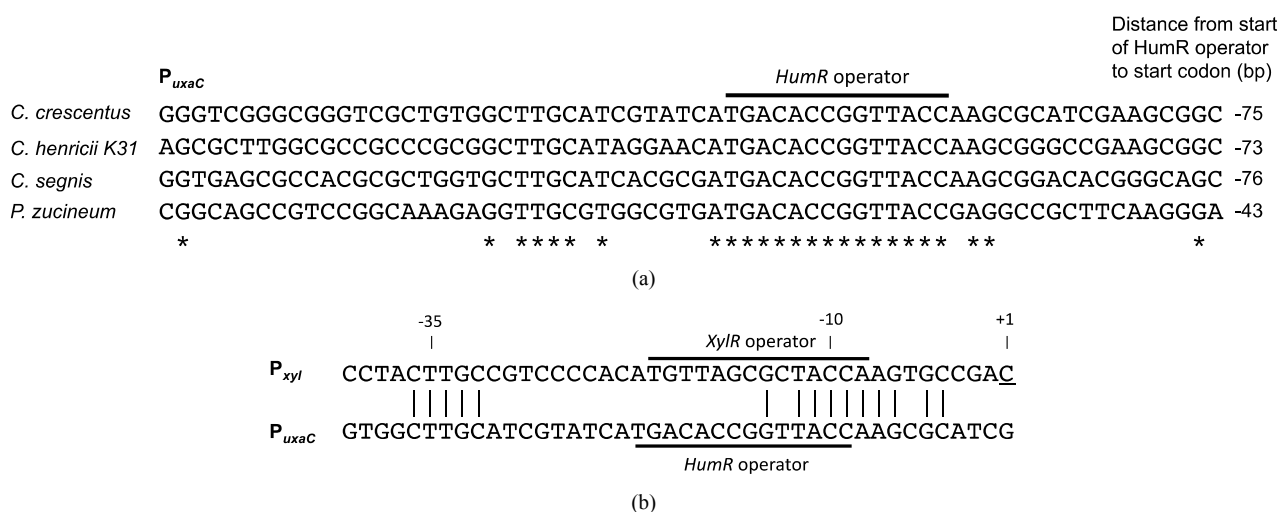


Figure 3. The *uxaC* promoter and HumR operator. a) Alignment of *uxaC* promoter region in *C. crescentus* and related species (*C. henricii* K31, *C. segnis* and *P. zucineum*). Distance is shown to the start codon, as transcription start sites could not be determined; b) Alignment of *C. crescentus* P_{xyl} [14] and P_{uxaC} . Numbering for P_{xyl} is shown relative to the transcription start site [14].

cies, which are members of the GntR class of transcription factors [5,33]. As in *C. crescentus*, the hexuronate regulon in *E. chrysanthemi* includes the hexuronate isomerase pathway, extracellular enzymes for pectin breakdown, and inner membrane transporters (ExuT) [34,35]. Neither *C. crescentus*, its close relatives, or the *Xanthomonas* species encode KdgR-like genes. Instead, *humR* is embedded within the hexuronate metabolic gene cluster in the genomes of close *C. crescentus* relatives (*C. segnis*, *C. henricii* strain K31, *Maricaulis maris*, and *Asticcacaulis excentricus*). *Phenylobacterium zucineum* has a modified cluster, with the HumR-regulated operon homologous to CC_1509-1508 (*exuT*) inserted between *humR* and *uxaC*. Based on the presence of HumR operators, the HumR regulon is largely conserved in these species, with the exception of CC_1446 and CC_3152, which are unique to *C. crescentus*. *Xanthomonas campestris* and *X. axonopodis*, which like *Erwinia chrysanthemi* are gamma-Proteobacterial phytopathogens [36], may also utilize a HumR-type regulatory system, as their genomes have a HumR homolog located immediately adjacent to *kduI* and *kduD*, and possible operators located upstream of the *kduID* operon and the *kdgK* gene (not shown).

Genomic clustering of the genes for hexuronate metabolism (CC_1487 – CC_1496) undoubtedly has facilitated their co-regulation, and potential to be horizontally transferred as a functional unit. The breakdown of synteny outside of this gene cluster is consistent with the hypothesis that horizontal transfer introduced this pathway into the *Caulobacter* lineage. The inclusion of a simple transcription control system in the cluster was likely beneficial in that it ensured appropriate regulation of the pathway in a new host. Price *et al.* [37] found in *E. coli* that most “neighbor regulators”—transcription factors that are genetically adjacent to the genes they regulate—were likely acquired by horizontal gene transfer with their target genes. A similar pattern applies to other-LacI-type repressors in the *Caulobacter* group, since the hexuronate, glucose, and maltose-responsive repressors are clustered with genes they control in the *C. crescentus* genome, and XylR is clustered with the *xyl* operon in the *C. henricii* K31 genome. Such clustering should help in elucidating the regulatory function of the many uncharacterized LacI homologs in the *Caulobacter* group.

5. CONCLUSION

Caulobacter crescentus encodes genes (CC_1487 to CC_1495) necessary for metabolism of galacturonate through the hexuronate isomerase pathway. A conserved 14 bp operator upstream of three operons within this cluster binds HumR, a LacI-type repressor encoded by the CC_1489 gene. HumR represses these promoters in the absence of galacturonate, and releases the operator in its

presence. Several additional genes likely involved in pectin degradation and hexuronate transport also appear to be part of the HumR regulon, which joins a growing roster of plant polysaccharide utilization systems controlled by LacI repressor homologs in *C. crescentus*.

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REFERENCES

- [1] Mohnen, D. (2008) Pectin structure and biosynthesis. *Current Opinion in Plant Biology*, **11**, 266-277. <http://dx.doi.org/10.1016/j.pbi.2008.03.006>
- [2] Abbott, D.W. and Boraston, A.B. (2008) Structural biology of pectin degradation by Enterobacteriaceae. *Microbiology and Molecular Biology Reviews*, **72**, 301-316. <http://dx.doi.org/10.1128/MMBR.00038-07>
- [3] Richard, P. and Hilditch, S. (2009) D-galacturonic acid catabolism in microorganisms and its biotechnological relevance. *Applied Microbiology and Biotechnology*, **82**, 597-604. <http://dx.doi.org/10.1007/s00253-009-1870-6>
- [4] Yadav, S., Yadav, P., Yadav, D. and Yadav, K. (2009) Pectin lyase: A review. *Process Biochemistry*, **44**, 1-10. <http://dx.doi.org/10.1016/j.procbio.2008.09.012>
- [5] Hugouvieux-Cotte-Pattat, N. and Robert-Baudouy, J. (1987) Hexuronate catabolism in *Erwinia chrysanthemi*. *Journal of Bacteriology*, **169**, 1223-1231.
- [6] Entcheva-Dimitrov, P. and Spormann, A.M. (2004) Dynamics and control of biofilms of the oligotrophic bacterium *Caulobacter crescentus*. *Journal of Bacteriology*, **186**, 8254-8266. <http://dx.doi.org/10.1128/JB.186.24.8254-8266.2004>
- [7] Poindexter, J. (1964) Biological properties and classification of the *Caulobacter* group. *Bacteriology Reviews*, **28**, 231-295.
- [8] Nierman, W.C., Feldblyum, T.V., Laub, M.T., Paulsen, I.T., Nelson, K.E., Eisen, J., *et al.* (2001) Complete genome sequence of *Caulobacter crescentus*. *Proceedings of the National Academy of Sciences of USA*, **98**, 4136-4141. <http://dx.doi.org/10.1073/pnas.061029298>
- [9] Mannisto, M.K., Tiirola, M.A., Salkinoja-Salonen, M.S., Kulomaa, M.S. and Puhakka, J.A. (1999) Diversity of chlorophenol-degrading bacteria isolated from contaminated boreal groundwater. *Archives of Microbiology*, **171**, 189-197. <http://dx.doi.org/10.1007/s002030050698>
- [10] Luo, Y., Xu, X., Ding, Z., Liu, Z., Zhang, B., Yan, Z., Sun, J., Hu, S. and Hu, X. (2008) Complete genome of *Phenylobacterium zucineum*, a novel facultative intracel-

- lular bacterium isolated from human erythroleukemia cell line K562. *BioMed Central Genomics*, **9**, 386. <http://dx.doi.org/10.1186/1471-2164-9-386>
- [11] Hottes, A.K., Meewan, M., Yang, D., Arana, N., Romero, P., McAdams, H.H. and Stephens, C. (2004) Transcriptional profiling of *Caulobacter crescentus* during growth on complex and minimal media. *Journal of Bacteriology*, **186**, 1448-1461. <http://dx.doi.org/10.1128/JB.186.5.1448-1461.2004>
- [12] Stephens, C., Christen, B., Fuchs, T., Sundaram, V., Watanabe, K. and Jenal, U. (2007) Genetic analysis of a novel pathway for D-xylose metabolism in *Caulobacter crescentus*. *Journal of Bacteriology*, **189**, 2181-2185. <http://dx.doi.org/10.1128/JB.01438-06>
- [13] Stephens, C., Christen, B., Watanabe, K., Fuchs, T. and Jenal, U. (2008) Regulation of D-xylose metabolism in *Caulobacter crescentus* by a LacI-type repressor. *Journal of Bacteriology*, **189**, 8828-8834. <http://dx.doi.org/10.1128/JB.01342-07>
- [14] Meisenzahl, A.C., Shapiro, L. and Jenal, U. (1997) Isolation and characterization of a xylose-dependent promoter from *Caulobacter crescentus*. *Journal of Bacteriology*, **179**, 592-600.
- [15] Ely, B. (1991) Genetics of *Caulobacter crescentus*. *Methods in Enzymology*, **204**, 372-384. [http://dx.doi.org/10.1016/0076-6879\(91\)04019-K](http://dx.doi.org/10.1016/0076-6879(91)04019-K)
- [16] Bochner, B.R. (2003) New technologies to assess genotype-phenotype relationships. *Nature Reviews Genetics*, **4**, 309-314. <http://dx.doi.org/10.1038/nrg1046>
- [17] West, L., Yang, D. and Stephens, C. (2002) Use of the *Caulobacter crescentus* genome sequence to develop a method for systematic genetic mapping. *Journal of Bacteriology*, **184**, 2155-2166. <http://dx.doi.org/10.1128/JB.184.8.2155-2166.2002>
- [18] Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research*, **31**, 3406-3415. <http://dx.doi.org/10.1093/nar/gkg595>
- [19] Stephens, C. and Shapiro, L. (1993) An unusual promoter controls cell-cycle regulation and dependence on DNA replication of the *Caulobacter fljLM* early flagellar operon. *Molecular Microbiology*, **9**, 1169-1179. <http://dx.doi.org/10.1111/j.1365-2958.1993.tb01246.x>
- [20] Hruz, T., Wyss, M., Docquier, M., Pfaffl, M., Masanetz, S., Borghi, L., *et al.* (2011) RefGenes: identification of reliable and condition specific reference genes for RT-qPCR data normalization. *BMC Genomics*, **12**, 156. <http://dx.doi.org/10.1186/1471-2164-12-156>
- [21] Lohmiller, S., Hantke, K., Patzer, S.I. and Braun, V. (2008) TonB-dependent maltose transport by *Caulobacter crescentus*. *Microbiology*, **154**, 1748-1754. <http://dx.doi.org/10.1099/mic.0.2008/017350-0>
- [22] Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods*, **25**, 402-408. <http://dx.doi.org/10.1006/meth.2001.1262>
- [23] Williams, K.P., Sobral, B. and Dickerman, A. (2007) A robust species tree for the Alphaproteobacteria. *Journal of Bacteriology*, **189**, 4578-4586. <http://dx.doi.org/10.1128/JB.00269-07>
- [24] Boer, H., Maaheimo, H., Koivula, A., Penttila, M. and Richard, P. (2009) Identification in *Agrobacterium tumefaciens* of the galacturonic acid dehydrogenase gene. *Applied Microbiology and Biotechnology*, **86**, 901-909. <http://dx.doi.org/10.1007/s00253-009-2333-9>
- [25] Riley, R.G. and Kolodziej, B.J. (1976) Pathway of glucose catabolism in *Caulobacter crescentus*. *Microbios*, **1**, 219-226.
- [26] Bailey, T., Bodén, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., *et al.* (2009) MEME SUITE: Tools for motif discovery and searching. *Nucleic Acids Research*, **37**, W202-W208. <http://dx.doi.org/10.1093/nar/gkp335>
- [27] Fischer, M., Zhang, Q.Y., Hubbard, R.E. and Thomas, G.H. (2010) Caught in a TRAP: Substrate-binding proteins in secondary transport. *Trends in Microbiology*, **18**, 471-478. <http://dx.doi.org/10.1016/j.tim.2010.06.009>
- [28] Haseloff, B.J., Freeman, T.L., Valmeekam, V., Melkus, M.W., Oner, F., Valachovic, M.S. and San Francisco, M.J. (1998) The *exuT* gene of *Erwinia chrysanthemi* EC16: Nucleotide sequence, expression, localization, and relevance of the gene product. *Molecular Plant-Microbe Interactions*, **11**, 270-276. <http://dx.doi.org/10.1094/MPMI.1998.11.4.270>
- [29] Collmer, A., Whalen, C., Beer, S. and Bateman, D. (1982) An *exo-poly- α -D-galacturonosidase* implicated in the regulation of extracellular pectatylase production in *Erwinia chrysanthemi*. *Journal of Bacteriology*, **149**, 626-634.
- [30] Swint-Kruse, L. and Matthews, K. (2009) Allostery in the LacI/GalR family: Variations on a theme. *Current Opinions in Microbiology*, **12**, 129-137. <http://dx.doi.org/10.1016/j.mib.2009.01.009>
- [31] Lin, J.-S. and Shaw, G.-C. (2007) Regulation of the *kduID* operon of *Bacillus subtilis* by the KdgR repressor and the *ccpA* gene: Identification of two KdgR-binding sites within the *kdgR-kduI* intergenic region. *Microbiology*, **153**, 701-710. <http://dx.doi.org/10.1099/mic.0.2006/002253-0>
- [32] Blanvillain, S., Meyer, D., Boulanger, A., Lautier, M., Guynet, C., Denanc, A.N., Vasse, J., Lauber, E. and Arlat, M. (2007) Plant carbohydrate scavenging through TonB-dependent receptors: A feature shared by phytopathogenic and aquatic bacteria. *PLoS One*, **2**, e224. <http://dx.doi.org/10.1371/journal.pone.0000224>
- [33] Hugouvieux-Cotte-Pattat, N., Condemine, G., Nassar, W. and Reverchon, S. (1996) Regulation of pectinolysis in *Erwinia chrysanthemi*. *Annual Review of Microbiology*, **50**, 213-257. <http://dx.doi.org/10.1146/annurev.micro.50.1.213>
- [34] Rodionov, D.A., Gelfand, M.S. and Hugouvieux-Cotte-Pattat, N. (2004) Comparative genomics of the KdgR regulon in *Erwinia chrysanthemi* 3937 and other gamma-proteobacteria. *Microbiology*, **150**, 3571-3590. <http://dx.doi.org/10.1099/mic.0.27041-0>
- [35] Rodionov, D.A., Mironov, A.A., Rakhmaninova, A.B. and Gelfand, M.S. (2000) Transcriptional regulation of transport and utilization systems for hexuronides, hexuronates and hexonates in gamma purple bacteria. *Molecular Microbiology*, **38**, 673-683. <http://dx.doi.org/10.1046/j.1365-2958.2000.02115.x>

- [36] Da Silva, A.C., Ferro, J.A., Reinach, F.C., Farah, C.S., Furlan, L.R., Quaggio, R.B., *et al.* (2002) Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature*, **417**, 459-463.
<http://dx.doi.org/10.1038/417459a>
- [37] Price, M.N., Dehal, P. and Arkin, A. (2008) Horizontal gene transfer and the evolution of transcriptional regulation in *Escherichia coli*. *Genome Biology*, **9**, R4.
<http://dx.doi.org/10.1186/gb-2008-9-1-r4>