

Implications of carbon catabolite repression for plant–microbe interactions

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Implications of carbon catabolite repression for plant–microbe interactions

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ABSTRACT

Carbon catabolite repression (CCR) plays a key role in many physiological and adaptive responses in a broad range of microorganisms that are commonly associated with eukaryotic hosts. When a mixture of different carbon sources is available, CCR, a global regulatory mechanism, inhibits the expression and activity of cellular processes associated with utilization of secondary carbon sources in the presence of the preferred carbon source. CCR is known to be executed by completely different mechanisms in different bacteria, yeast, and fungi. In addition to regulating catabolic genes, CCR also appears to play a key role in the expression of genes involved in plant–microbe interactions. Here, we present a detailed overview of CCR mechanisms in various bacteria. We highlight the role of CCR in beneficial as well as deleterious plant–microbe interactions based on the available literature. In addition, we explore the global distribution of known regulatory mechanisms within bacterial genomes retrieved from public repositories and within metatranscriptomes obtained from different plant rhizospheres. By integrating the available literature and performing targeted meta-analyses, we argue that CCR-regulated substrate use preferences of microorganisms should be considered an important trait involved in prevailing plant–microbe interactions.

Key words: carbon catabolite repression, CCR, carbon utilization, plant–microbe interaction, rhizosphere, substrate-use preferences

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INTRODUCTION

Plants are usually inhabited by a large number of different microorganisms. The interactions within the microbial community and the plant host are driven by different survival strategies of the organisms involved. For instance, some microorganisms may live in close symbiotic associations and are thus reliant on distinct partners in the plant holobiont. Conversely, others may engage in ferocious competition, resulting in a relentless war to win finite resources such as nutrients, light, or territory (Bauer et al., 2018). Competition is often linked to the acquisition of nutritional resources that contribute to the improved development of the organism. To ensure their survival in the presence of competitors, some organisms have developed specific strategies that enable them to gain an advantage over others. Prominent examples of such adaptations are bacteria from the genus *Pseudomonas*, which are known to frequently occur in the plant rhizosphere (Haichar et al., 2012) and to produce siderophores with various affinities as well as receptors for xenosiderophores produced by other species, thereby ensuring the efficient acquisition of iron (Taguchi et al., 2010). While the efficient acquisition of nutrients is a crucial prerequisite for microbial development, microorganisms are also able to make use of their highly versatile metabolic capacities. This allows them to fulfil their nutritional needs via a wide range of resources present in the environment. However, this functional versatility comes with high energetic costs for the cell (Carlson and Taffs, 2010). To reduce the effects of such energy constraints, microorganisms have developed a variety of metabolic regulatory mechanisms. This allows them to thrive at the lowest possible cost. Catabolic regulation primarily involves

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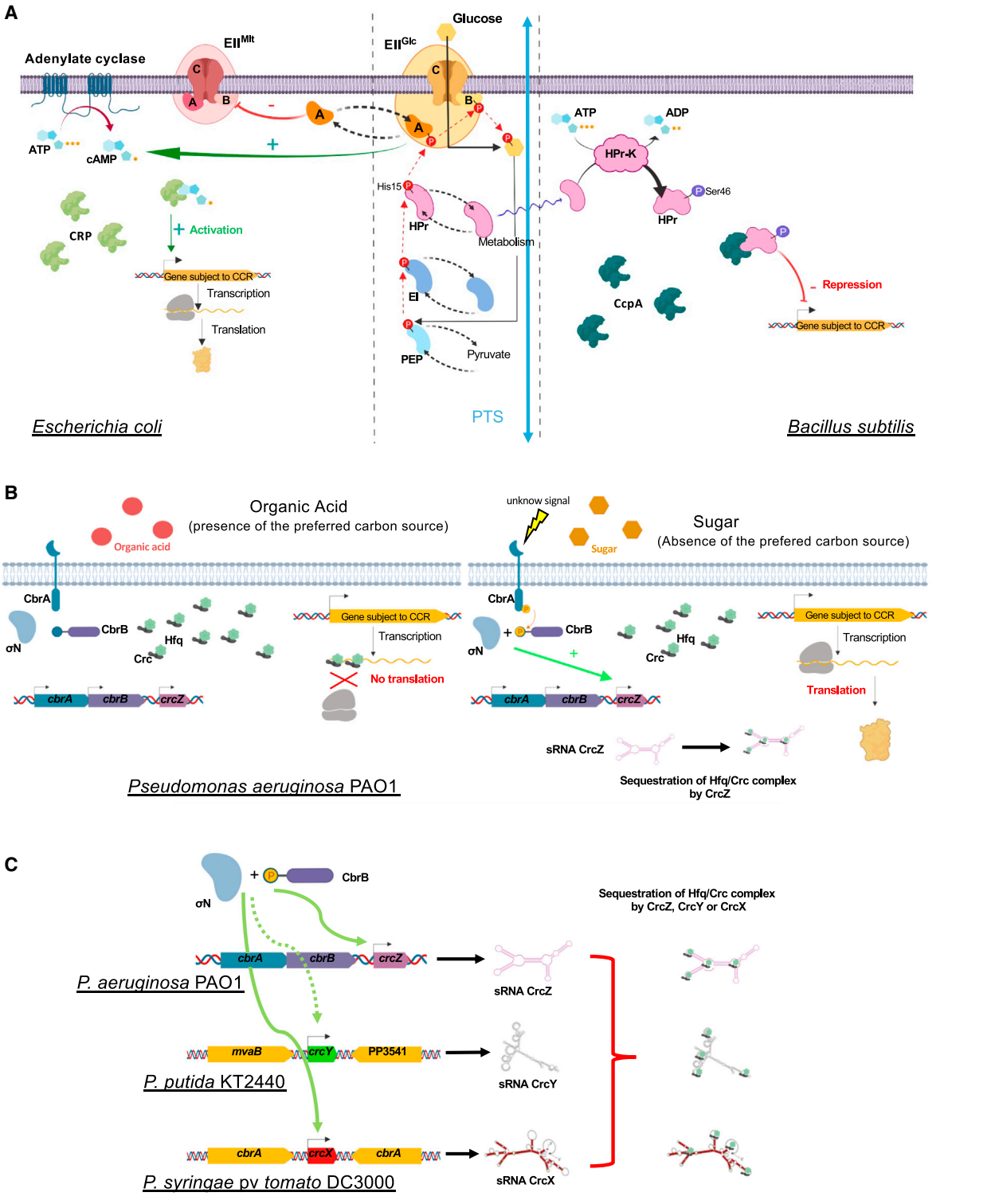


Figure 1. Schematic representation of the CCR system in Enterobacteriaceae (*E. coli*) and Firmicutes (*B. subtilis*) and of the revCCR system in pseudomonads.

(A) The PTS phosphorylation cascade allows the transport and metabolism of different sugars within the cell. The PTS consists of a set of phospho-transferases, which can include enzyme I (EI), histidine-containing protein (HPr), and phosphotransferases like enzyme II (EII). The EII subunits exhibit different localizations (EIIA and EIIB, cytoplasmic; EIIC and EIID, transmembrane). EII enzymes are substrate specific and thus synthesized only in the presence of the substrate. The first reaction step of this system is the phosphorylation of EI by phosphoenolpyruvate (PEP) (Alpert et al., 1985); phosphorylation is then transferred from protein to protein (from EI to HPr at the His-15 position to EIIA) to the B subunit of EII^{Glc}, which, once phosphorylated, allows the transport of glucose from the outside to the inside of the cell via the C subunit. For Enterobacteriaceae, inducer exclusion

(legend continued on next page)

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directing available energy into a distinct metabolic pathway that facilitates utilization of the available resource that yields the most energy in return. In short, catabolic regulation is generally directed toward providing the best return on investment.

The best-studied catabolic regulatory mechanism in bacteria is arguably carbon catabolite repression (CCR); this mechanism enables the preferential assimilation of one carbon source rather than another (Monod, 1949; Deutscher, 2008). For this reason, when glucose and lactose are present in equal amounts, *Escherichia coli* first metabolizes the glucose and only later the lactose present in the local environment. Following this crucial observation, numerous studies focused on catabolic repression in different organisms (Deutscher, 2008; Görke and Stülke, 2008; Rojo, 2010; Iyer et al., 2016). To date, two different CCR types have been discovered. Classical CCR regulates the preference for sugars, such as glucose, not only in Enterobacteriaceae and Firmicutes but also in eukaryotes such as yeasts and filamentous fungi (Deutscher, 2008; Görke and Stülke, 2008). Reverse CCR (revCCR) determines the preference of organic acids, such as succinate, in pseudomonads and rhizobia (Rojo, 2010; Iyer et al., 2016). revCCR has mainly been studied in *Pseudomonas aeruginosa* PAO1, which shows post-transcriptional repression, whereas classic CCR is regulated by transcriptional repression (Rojo, 2010). It is not fully clear whether, in addition to regulating catabolic genes, CCR can also control other genes. Moreno et al. (2001) suggested that 5%–10% of bacterial genes are subject to CCR regulation, including those involved in virulence or biofilm formation (Zheng et al., 2004; Rojo, 2010; Chen et al., 2020).

In this review, we address the central role of CCR in plant–microbe interactions in the rhizosphere. First, we provide a condensed overview of the different CCR mechanisms that have been characterized to date in various bacterial models. Then we continue to focus on microbial functions that are involved in interactions with plants and regulated by CCR. In addition, we perform a meta-analysis using available genomic and metatranscriptomic datasets to investigate the distribution of genes involved in bacterial catabolic repression within the plant rhizosphere. The results support our initial hypothesis that this mechanism is widespread among bacteria, specifically rhizobacteria. We use these new insights to discuss the ecological and environmental importance of CCR mechanisms in the rhizosphere.

MECHANISM OF CARBON CATABOLITE REPRESSION IN BACTERIA

In this first section, we focus on mechanistic aspects of CCR that can be found in different bacteria, such as members of Enterobacteriaceae (*E. coli*), Firmicutes (*Bacillus subtilis*), Rhizobia, and pseudomonads (*P. aeruginosa*). In general, CCR in bacteria is directly linked to carbohydrate transport into the cell. Notably, this system also acts as a regulatory system for nutrient uptake and certain physiological processes, such as virulence and biofilm formation (Görke and Stülke, 2008). Most of the mechanisms have been described using isolated microbes under laboratory conditions. However, it is likely that many of these mechanisms also play an important role in some host-metabolite-dominated environments, such as the plant rhizosphere. The mechanisms of CCR differ substantially among bacteria. Glucose and other sugars are the preferred carbon sources of enteric, Gram-positive bacteria and Firmicutes, the mechanism referred to as CCR, whereas organic acids are the repressing carbon sources in pseudomonads and rhizobia. The latter mechanism is commonly referred to as revCCR; we propose the term "organic acid-mediated CCR" for increased clarity.

CCR mechanisms in Enterobacteriaceae

In Enterobacteriaceae, such as *E. coli*, the main CCR regulator is known as glucose-specific component EIIA ($EIIA^{Glc}$). EIIA is a component of the phosphotransferase system (PTS) with phosphotransferases like enzyme I (EI), a histidine-containing protein (HPr), and different phosphotransferases (EII) (Figure 1A1). Depending on the phosphorylation state of $EIIA^{Glc}$, CCR is either activated or inactivated. In the presence of glucose, $EIIA^{Glc}$ is in a non-phosphorylated state and cannot stimulate adenylate cyclase (CyaA), but it can inhibit the transport of several non-PTS sugars by interacting with their transporter; this is known as induced exclusion (IE). IE can be considered a crucial component of CCR in Enterobacteriaceae and also in Firmicutes, but it involves different mechanisms in the latter. One of the best examples of IE in *E. coli* is the repression of the lac operon, in which $EIIA^{GLc}$ binds to the LacY transporter of lactose, preventing the uptake of the sugar and therefore the formation of allolactose required for dismissal of the lac repressor. In addition, CyaA is stimulated by P-$EIIA^{Glc}$ and results in cAMP production (Figure 1A) (Harwood et al., 1976; Feucht and Saier, 1980). cAMP can bind to and activate the cAMP receptor protein (Crp) as the major transcriptional activator of CCR (Figure 1A). Crp-cAMP then activates the transcription of different catabolic genes

is caused by the phosphorylation state of the $EIIA^{Glc}$ subunit. When $EIIA^{Glc}$ is not phosphorylated, i.e., during glucose transport, $EIIA^{Glc}$ interacts with and inactivates enzymes and transporters of secondary carbon sources. CCR is therefore active. Conversely, when $EIIA^{Glc}$ is phosphorylated, i.e., in the absence of glucose transport, $EIIA^{Glc}$ interacts with and activates adenylate cyclase for cAMP formation from ATP. Once formed, cAMP interacts with the cAMP receptor protein (Crp) and allows the transcription of genes associated with non-preferred carbon sources (and other functions that are also regulated) because the repression has been lifted. For Firmicutes, the CCR is orchestrated by the protein HPr. HPr can be phosphorylated at serine 46 by HPr-kinase (Hpr-K). The CcpA-Hpr–Ser-46 complex represses the transcription of genes associated with non-preferred carbon sources (and other functions that are also regulated).

(B) For pseudomonads, in the presence of the preferred carbon source (succinate), free Crc protein coupled with Hfq binds to the mRNAs of genes associated with non-preferred carbon sources (and other functions that are also regulated), thus preventing proper ribosome binding and mRNA translation. In the absence of the preferred carbon source, an unknown signal is transmitted to the CbrAB two-component system, which, together with the σ^N factor, allows the transcription of the small CrcZ non-coding RNA. CrcZ traps the Crc/Hfq couple, thus freeing the ribosome binding site of the Crc-regulated mRNAs. mRNAs are translated again, and repression is therefore lifted.

(C) Other small non-coding RNAs can lift the repression in other *Pseudomonas* species. Green arrows indicate positive control of RNA synthesis from CbrB. The green dotted arrow highlights a weaker control of CbrB on CrcY in *Pseudomonas putida*.

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and other operons. Several studies show that Crp-cAMP regulates the transcription of numerous *E. coli* genes (Shimada et al., 2011), suggesting a more complex role for this regulator than just CCR, such as involvement in biofilm formation and virulence in Enterobacteriaceae (El Mouali et al., 2018).

CCR mechanisms in Firmicutes

In Firmicutes, such as *B. subtilis*, HPr is the main regulator of CCR, by analogy to $EIIA^{Glc}$ in *E. coli*. The uptake of a preferred carbon source mediates the phosphorylation state of HPr through the bifunctional enzyme HPr-kinase/phosphorylase (HPr-K/P). HPr is phosphorylated on Ser-46 by HPr-K/P after being activated by glycolysis following glucose uptake (Mijakovic et al., 2002; Nessler et al., 2003) (Figure 1A). P-Ser-HPr can then bind to catabolic control protein A (CcpA) and repress the transcription of other catabolic genes by binding to them (Schumacher et al., 2004). The P-Ser-HPr/CcpA complex binds to DNA at a specific promoter region called the catabolic-response element (*cre*) to inhibit transcription of the targeted gene (Miwa et al., 2000) (Figure 1A). For instance, CcpA negatively regulates ribose transporter genes or *chiB*, a gene necessary for chitinase production in *Bacillus thuringiensis* (Antunes et al., 2012; Jiang et al., 2015), in the presence of glucose. In addition, CcpA can positively regulate some genes required for acetate production, like *ackA* and *pta*, by binding to their upstream promoter regions (Presecan-Siedel et al., 1999). Other catabolic control proteins, including CcpB, CcpC, and CcpN, have analogous regulatory functions (Chauvaux et al., 1998; Kim et al., 2003; Servant et al., 2005).

CCR mechanisms in rhizobia

Carbohydrate transport in rhizobia differs from that in Enterobacteriaceae and Firmicutes, as carbon transport is not mediated by PTS. For example, fructose enters *Rhizobium leguminosarum* in an unphosphorylated form, whereas mannose is phosphorylated by the mannose uptake system (Arias et al., 1982; Glenn et al., 1984). In rhizobia, the transport of some organic acids, like succinate, is orchestrated by a dicarboxylate transport system encoded by *dct* genes (Ronson et al., 1987; Watson et al., 1993). In contrast to their utilization by the aforementioned bacteria, sugars are secondary carbon sources for rhizobia, and organic acids are preferred (Ucker and Signer, 1978); thus, their catabolic repression is named revCCR or succinate-mediated catabolite repression (SMCR), as described by Mandal and Chakrabartty (1993). Several studies have shown that succinate and other organic acids repress the activity of sugar transporters and major metabolic pathways like Entner–Doudoroff and Embden Meyerhof Parnas (Arias et al., 1982; Mukherjee and Ghosh, 1987). Interestingly, *Sinorhizobium meliloti* possesses the PTS protein Hpr by analogy to Enterobacteriaceae and the kinase/phosphatase HprK as found in Gram-positive bacteria. According to Pinedo et al. (2008), Hpr plays a role in SMCR, as an *hpr* mutant showed a decrease in SMCR for raffinose but not for lactose. We still lack a detailed understanding of SMCR regulation in rhizobia in terms of how organic acids induce repression and which regulators are implemented (Iyer et al., 2016). revCCR has been described mainly for *Pseudomonas*, in which the fine-tuning of repression is well described, even though the system appears to be different.

CCR mechanisms in pseudomonads

Sugar uptake in different members of the genus *Pseudomonas* is mediated by ABC transporters, such as the GtsA protein involved in glucose transport in *Pseudomonas stutzeri* A1501 (Liu et al., 2020), and by outer and inner membrane proteins (Raneri et al., 2018). Moreover, organic acids are the preferred carbon source for pseudomonads. Consequently, their CCR is considered revCCR (Collier et al., 1996; Rojo, 2010). revCCR, or organic acid–mediated CCR, is mediated at a post-transcriptional level; the regulation is orchestrated by the master regulator protein catabolic repression control (Crc). Crc first stabilizes the interaction between Hfq and mRNA and subsequently stabilizes the post-transcriptional repression of genes associated with the use of a non-preferred carbon source through the inability of the ribosome to bind to mRNA and initiate translation (Sonnleitner et al., 2012, 2017, 2018; Sonnleitner and Bläsi, 2014) (Figure 1B). Indeed, Hfq was first described in Gram-negative bacteria as a global post-transcriptional regulator based on its RNA binding properties (Sonnleitner and Bläsi, 2014). Thus, under organic acid–mediated CCR conditions, translation of aliphatic amidase(*amiE*), autotransporter esterase (*estA*), and phenazine-specific methyltransferase (*phzM*) mRNAs is repressed by the Crc/Hfq complex in *P. aeruginosa* PAO1 (Sonnleitner et al., 2012). In addition, the two-component system (TCS) CbrAB, which belongs to the NtrBC family, was found to regulate the assimilation of C sources, but its activation is still unclear. CbrAB and NtrBC (a TCS that enables assimilation of nitrogen sources) have been found to act together to maintain a C/N balance (Nishijyo et al., 2001; Li and Lu, 2007; Naren and Zhang, 2021). CbrAB and NtrBC TCSs have been shown to participate in a CCR mechanism for histidine utilization in *Pseudomonas fluorescens* SBW25 by interacting with each other to maintain C/N homeostasis (Naren and Zhang, 2021).

In the absence of the preferred carbon source, the repression is lifted. The CbrAB TCS induces the expression of CrcZ, a small RNA (sRNA), with the help of the sigma factor RpoN. CrcZ has different binding sites with high affinity for Crc. CrcZ sequesters Crc, and thus the Crc/Hfq complex can no longer bind to the mRNA, and the repression is no longer effective (Sonnleitner et al., 2017) (Figure 1B). Interestingly, some *Pseudomonas* species have more than one sRNA that can control the level of free Crc. Whereas *P. aeruginosa* possesses only CrcZ, *Pseudomonas syringae* and *Pseudomonas putida* possess CrcZ/CrcX and CrcZ/CrcY, respectively (Moreno et al., 2012; Filiatrault et al., 2013) (Figure 1C). Their functions seem to be redundant, although their regulation may differ between species. Moreover, CbrAB appears to regulate CrcZ and CrcX transcription in *P. syringae* pv. tomato DC300 (Filiatrault et al., 2013), but it has little effect on CrcY transcription in *P. putida* (Moreno et al., 2012) (Figure 1C).

BENEFICIAL AND DELETERIOUS CCR-MEDIATED INTERACTIONS BETWEEN MICROBES AND PLANTS

Microorganisms associated with distinct plants not only rely on adaption mechanisms that are required in the local environment but also must adapt to the metabolic activity of other members of the microbiota as well as the host plant itself. It is known that 5%–10% of all bacterial genes are subject to CCR

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(Görke and Stülke, 2008; Rojo, 2010). We argue that a certain proportion of these genes participate in the regulation of interactions within the plant holobiont. These regulations can be either direct, such as by improving plant growth, or indirect, by shielding plants from pathogens. The latter effect can be achieved if CCR affects competition between beneficial microorganisms and pathogens. On the other hand, pathogens also rely on various CCR mechanisms when infecting their hosts. A non-exhaustive list of the different functions regulated by Crc, CcpA, and Crp that are involved in nutrient acquisition or other relevant plant–bacteria interactions is presented in Table 1. Specific CCR-related functions were selected and are subjected to a deeper review below.

Improved use of carbon sources during N fixation or root exudate utilization is regulated by the CCR system

In the free-living (N)-fixing bacterium *Azotobacter vinelandii*, CCR occurs under both diazotrophic and non-diazotrophic conditions (Quiroz-Rocha et al., 2017; Martínez-Valenzuela et al., 2018). For example, the preference for acetate assimilation over glucose has been shown in *A. vinelandii* grown under N-fixing conditions (Tauchert et al., 1990). Quiroz-Rocha et al. (2017) provided evidence for the role of CbrAB and Crc/Hfq in the control of CCR processes, specifically the catabolic repression of glucose consumption, under diazotrophic conditions. *A. vinelandii* imports glucose using a GluP transporter (annotated as an H^+-coupled glucose–galactose symporter), a protein that is absent in most *Pseudomonas* spp. Levels of *gluP* mRNA were shown to be reduced in the presence of acetate and increased 19-fold during glucose uptake. Moreover, *gluP* mRNA levels were reduced when Crc was overexpressed, suggesting that *gluP* may be one of the targets of Crc-Hfq for CCR control. Furthermore, Martínez-Valenzuela et al. (2018) demonstrated that CCR is also controlled by CbrAB and Crc/Hfq under non-diazotrophic conditions.

The plant-growth-promoting bacterium *P. fluorescens* SBW25 can grow on different substrates, including xylose. Xylose is the backbone monomer of hemicellulose, which is a major structural component of the plant cell wall. Xylose also accumulates on plant surfaces colonized by microorganisms and has been identified as a dominant constituent of root exudates in a wide range of plant species (Guyonnet et al., 2017). Many plant-associated bacteria such as *P. fluorescens* SBW25 are able to grow on xylose as a carbon and energy source (Zhang and Rainey, 2008; Liu et al., 2015). In an interesting study, Liu et al. (2017) performed transposon mutagenesis of Δ*cbrB* and selected suppressors of xylose utilization (*xut* gene). They provided evidence that Crc/Hfq sequentially binds (and represses) transcriptional activator mRNAs and structural genes involved in xylose catabolism. However, in the absence of succinate, repression is relieved through the competitive binding of two sRNAs, CrcY and CrcZ, whose expression is activated by CbrAB.

Plant-colonizing bacteria continually alter gene expression patterns, particularly those involved in nutrition acquisition, as root exudation is dynamic (Haichar et al., 2014). Interestingly, among *P. fluorescens* SBW25 genes with elevated expression during plant colonization, Gal et al. (2003) and Giddens et al. (2007) found implications of *dctA* for succinate uptake and *xutA* for xylose catabolism. These findings strongly implicate succinate and xylose as two carbon substrates frequently encountered *in planta*. Liu et al. (2017) demonstrated that a wild-type strain in which CCR is intact outcompeted a CCR-defective strain when succinate was present at concentrations above 20 μM; but more significantly, it imposed a fitness burden under conditions of low succinate (<20 μM). These results suggest that the ecological significance of CCR depends on fluctuating concentrations of the preferred carbon source and may confer a selective disadvantage when succinate is present at low concentrations together with xylose.

Catabolic repression is involved in symbioses with legumes for nitrogen fixation

The ability to utilize a broad range of carbon sources is an important trait that allows adaptability to growth habitats. Rhizobia are found in various associations with plants, as well as in the soil as free-living organisms. To fix nitrogen, these bacteria can utilize the carbon sources available in the plant's root nodules. In exchange, the bacteria provide a constant supply of ammonia to the plant (O'Gara et al., 1989). C4-dicarboxylates, the preferred C source for rhizobia, appear to play a central role in symbiosis. They are the major C source provided to bacteroids by the host plant. These compounds have been shown to support the highest levels of nitrogen fixation in isolated bacteroids *in vitro*. Relevant work on soybean, pea, alfalfa, and lupine bacteroids showed that N_2 fixation in isolated bacteroids was highly stimulated by C4-dicarboxylic acids but not by sucrose (Yurgel and Kahn, 2004). Moreover, C4-dicarboxylates are also chemoattractants for rhizobia. Mutants with defective dicarboxylate transporters generally retain the ability to nodulate a host plant, but the nodules formed are unable to fix nitrogen (Ronson et al., 1981; Arwas et al., 1985). In addition, successful establishment of a symbiosis via penetration of the infection thread by rhizobia requires both continuous biosynthesis of Nod factors and biosynthesis of symbiotic exopolysaccharide (EPS) (Jones et al., 2007). EPS-deficient mutants of *S. meliloti* either fail to nodulate their host plants or induce the formation of ineffective nodules (Leigh et al., 1985; Rolfe et al., 1996; Skorupska et al., 2006). Among the known EPS molecules, succinoglycan plays a critical role in the *S. meliloti* symbiosis with alfalfa (Cheng and Walker, 1998; Mendis et al., 2016). Mutations in components of the PTS-like system (*hpr* and *eIIA*) have shown negative effects on EPS production (Pinedo et al., 2008). Deletion of HPr led to impaired control of succinoglycan synthesis. By contrast, a ΔmanX (an $EIIA^{Man}$-type enzyme) strain showed accumulation of high-molecular-weight succinoglycan. In addition to negative effects on EPS production, *manX* null mutants also showed an inability to grow using different carbon sources (Bélanger et al., 2009) and exhibited lower expression levels of genes involved in raffinose and lactose utilization (Bringhurst and Gage, 2002). It therefore seems that, depending on the nature of the carbon source exuded by leguminous plants, the SMCR system of rhizobia can be activated to induce EPS production, which is indispensable for successful infection of the host plant roots leading to ammonia production and hence to plant nutrition.

Catabolic repression mediates host protection against pathogens

In addition to regulation of the primary metabolite, CCR also regulates the production of secondary metabolites involved in

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	Bacteria versus plant	Gene/sRNA targeted	Regulatory mechanism	Physiological effect	References
Metabolism/ nutrition	*Pseudomonas putida*	Crc	Crc regulates the expression of branched-chain keto acid dehydrogenase	Complex branched-chain keto acid dehydrogenase	Hester et al. (2000)
	P. putida GPo1	Crc	Crc reduces the expression of the AlkS regulator, which activates alkane degradation	Alkane degradation	Yuste and Rojo (2001)
	Pseudomonas fluorescens SBW25	Hfq/Crc/sRNA and CbrAB	Crc/Hfq sequentially bind to (and repress) mRNAs of both the transcriptional activator and the structural genes involved in xylose catabolism; in the absence of succinate, repression is relieved through competitive binding by two ncRNAs, CrcY and CrcZ, whose expression is activated by CbrAB	Xylose and histidine utilization	Liu et al. (2017) and Zhang and Rainey (2008)
	Azotobacter vinelandii	CbrA/CbrB, Hfq, Crc, CrcZ, and CrcY	Crc-Hfq proteins recognize the *gluP* A-rich Hfq-binding motif, reducing translation in a Crc-dependent manner; CrbB and CrcZ/Y are essential for GluP expression	Glucose uptake through GluP transporter	Quiroz-Rocha et al. (2017) and Martínez-Valenzuela et al. (2018)
Symbiosis	*Sinorhizobium meliloti* versus alfalfa	Hpr and EIIAMan-type enzyme	Hpr and EIIAMan-type enzyme positively regulate EPS production; EIIAMan-type enzyme positively regulates growth using different carbon sources: succinate, glucose, glycerol, raffinose, lactose, and maltose	Mutants unable to transport C4 dicarboxylic acids are able to nodulate plants, but the bacteroids do not fix N; mutations in components of the PTS-like system (Hpr and EIIAMan-type enzyme) showed dramatic effects on EPS production	Finan et al. (1983), Ronson and Astwood (1985), Bélanger et al. (2009), and Pinedo et al. (2008)
Antibiotic and antifungal compounds	*Pseudomonas chlororaphis*	ND	ND	Phenazine 1-carboximide production using L-pyroglutamic acid and glucose; fructose, sucrose, and ribose repress phenazine 1-carboximide production	Van Rij et al. (2004)

Table 1. Carbon catabolite repression–mediated gene regulation in various bacterial and fungal species during interactions with plants.

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	Bacteria versus plant	Gene/sRNA targeted	Regulatory mechanism	Physiological effect	References
	P. fluorescens F113	ND	ND	Sucrose, fructose, and mannitol promoted high yields of 2,4-diacetylphloroglucinol (DAPG) by strain F113, whereas glucose and sorbose resulted in very poor DAPG production	Shanahan et al. (1992)
	Streptomyces coelicolor	cAMP-CRP system	*cya* mutants and strains defective in CRP showed a lack of germination and actinorhodin production	Spore germination and actinorhodin antibiotic production	Süsstrunk et al. (1998)
	Streptomyces griseus	ND	Glucose suppresses streptomycin production by mannosido streptomycinase repression	Spore germination and actinorhodin antibiotic production	(Demain and Inamine, 1970)
Biofilm formation	*Bacillus subtilis*	CcpA[a]	The expression of the gan operon was significantly induced in ccpA mutants; a putative high-affinity *cre* box was identified within the ganS promoter region	CcpA regulates the galactan utilization pathway, which results in production of UDP-Gal and UDP-Glu, two sugar nucleotides that are essential precursors for EPS biosynthesis during biofilm formation	Stanley et al. (2002), Marciniak et al. (2012), and Habib et al. (2017)
	Pseudomonas syringae pv. tomato DC3000 versus *Arabidopsis thaliana*	Crc	Crc inhibits biofilm formation	The △ crc strain showed enhancement of biofilm formation compared with the wild-type strain	Chakravarthy et al. (2017)
Virulence	*P. syringae* pv. tomato DC3000	Crc, CrcZ, CrcX	During plant infection, when T3SS is active, the expression of CrcZ and CrcX supports utilization of fructose and citrate (poor C sources)	Sugars such as glucose, sucrose, and fructose are known to be inducers of the *P. syringae* TTSS genes, whereas tricarboxylic acid intermediates can suppress T3SS *in vitro*; fructose and citrate utilization pathways are upregulated when cells are exposed to tomato apoplast extracts	Rico and Preston (2007), Chakravarthy et al. (2017), and Filiatrault et al. (2013)
	Dickeya dadantii versus chicory leaves	Crp–AMPc system	CAMP-CRP positively regulate pectate lyase production, virulence, and pathogenicity	*crp* mutation has serious consequences for the virulence of *D. dadantii*, as it strongly decreases pectate lyase production	Reverchon et al. (1991), Nasser et al. (1994), Hugouvieux-Cotte-Pattat (2016), Nasser et al. (1997), and Reverchon et al. (1997)

Table 1. *Continued*

(Continued on next page)

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	Bacteria versus plant	Gene/sRNA targeted	Regulatory mechanism	Physiological effect	References
	Xanthomonas campestris versus Chinese	HprK	HprK regulates genes that make a positive contribution to virulence, extracellular polysaccharides, extracellular enzymes, motility, and stress tolerance	Deletion of *hprK* demonstrated its requirement for virulence and other associated diverse cellular processes, including extracellular enzyme activity, extracellular polysaccharide production, and cell motility	Li et al. (2019)
Quorum sensing	*D. dadantii* 3937	Crp–AMPc system	The cAMP–Crp system induces an increase in AHL production when less of the preferred substrate is present; CRP activates expR expression and represses expI transcription	CRP induces virulence	Nasser et al. (1998) and Reverchon et al. (1998)

Table 1. *Continued*

AHL, N-acyl-homoserine lactone; EPS, exopolysaccharide; T3SS, type III secretion system; ND, not determined.

host–microbe and microbe–microbe interactions. Various rhizobacteria produce bioactive compounds that can protect plants against disease and thus improve plant health. The production of antifungal and antibacterial metabolites is also considered an important prerequisite for optimal performance of biocontrol agents (Chin-A-Woeng et al., 2003). *Pseudomonas* species have been shown to commonly produce several types of antifungal compounds, the most studied of which are pyoluteorin, pyrrolnitrin, phenazines, and 2,4-diacetylphloroglucinol (DAPG) (Chin-A-Woeng et al., 2003). In several *Pseudomonas* species, phenazine production is affected by the available carbon source. For example, Van Rij et al. (2004) tested the effects of different sources of carbon present in tomato root exudates on phenazine production in a *Pseudomonas chlororaphis* strain. The highest production of phenazine 1-carboximide was obtained in the presence of L-pyroglutamic acid and glucose. However, fructose, sucrose, and ribose had negative effects on phenazine 1-carboximide production. Similar results were observed in a *gacA*-inactivated *Pseudomonas* sp. M18G, in which the highest production of phenazine 1-carboxylic acid was achieved with glucose and ethanol as carbon sources (Li et al., 2008). Moreover, DAPG production has also been shown to be regulated by the carbon source in *P. fluorescens F113* (Shanahan et al., 1992). Sucrose, fructose, and mannitol promoted high yields of DAPG, whereas glucose and sorbose resulted in drastically reduced DAPG production. Thus, catabolic repression in *Pseudomonas* species inhabiting the plant rhizosphere may play a crucial role in the expression of genes involved in the control of plant root diseases, depending on the nature of exudates released by the plants during plant–bacteria interactions.

The production of secondary metabolites, including antibiotics and bioactive compounds, is one of the main characteristics of members of *Streptomyces*. Glucose is generally the preferred carbon source for *Streptomyces* growth. However, when used in high concentrations, it also interferes with the formation of secondary metabolites (Demain, 1989). For example, glucose suppresses streptomycin production by *Streptomyces griseus*, known for plant growth promotion, by repressing mannosidostreptomycinase (Demain and Inamine, 1970). In addition, production of the actinorhodin antibiotic has been shown to depend on the cAMP-CRP system in the filamentous soil bacterium *Streptomyces coelicolor* (Süsstrunk et al., 1998).

The roles of catabolic repression in biofilm formation

Biofilms are structured communities of microorganisms in which cells are embedded in an extracellular matrix (Flemming and Wuertz, 2019), generally formed to promote bacterial survival in harsh environments (Flemming et al., 2016). Although biofilm formation is a complex process regulated by several different factors in various bacteria, CCR seems to be an important global regulator involved in biofilm production. Plant-beneficial representatives of *B. subtilis* can utilize polysaccharides and other carbohydrate substances present in the rhizosphere as major carbon sources, many of which are derived from the decomposition of plant tissues (Ochiai et al., 2007). Plant polysaccharides have been shown to stimulate the formation of root-associated multicellular communities, or biofilms. In *B. subtilis,* a five-gene *gan* operon (*ganSPQAB*) has been shown to participate in the utilization of galactan, a plant-derived polysaccharide (Habib et al., 2017). Interestingly, plant-derived galactan was shown to promote biofilm formation in *B. subtilis* (Habib et al., 2017). A putative high-affinity *cre* box was identified in the promoter region of *ganS*, with strong repression upon CcpA induction (Marciniak et al., 2012). In addition, by quantifying biofilm formation at

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different glucose concentrations, Stanley et al. (2003) showed that biofilm formation by *B. subtilis* is repressed by catabolites through the transcription factor CcpA. The same observation was also made in *P. syringae* DC3000; a Δ*crc* mutant showed enhanced biofilm formation compared with the wild-type strain (Chakravarthy et al., 2017). *P. syringae* is considered to be a hemibiotrophic pathogen that can cause diseases in both tomato and *Arabidopsis*. When conditions are optimal, the bacteria invade plant tissues via wounds or natural openings like stomata, colonize the apoplastic space, and metabolize host nutrients in order to multiply and survive. The observed increase in biofilm formation may be detrimental for the *crc* mutant once it is inside the plant cell because of its inability to spread within the plant and hence grow and infect the plant.

Catabolic repression mediating quorum sensing regulation

Bacterial species employ a complex communication mechanism termed quorum sensing (QS) that is used to link cell density to gene expression. In this process, bacteria secrete chemical signaling molecules, called autoinducers (AIs), that accumulate as cell density increases. Once the AI level reaches a threshold, signaling a ''quorum'' of cells, AI signals are transported into cells, where they activate gene expression and enable coordinated phenotypic responses in the population (Ha et al., 2018). The QS system contributes to different processes in plant–microbe interactions, such as cell density maintenance, biofilm formation, antibiotic production, natural competence, sporulation, and pathogenicity (Pena et al., 2019). Some rhizobacteria and phytopathogenic bacteria have been shown to express several important genes under QS control. Few studies have focused on how the cell regulates QS processes, for example, based on the availability of substrates like glucose, which provides a link between CCR and QS. The most-studied model is *E. coli*, in which there is evidence that the QS system mediated by the autoinducer AI-2 is partially regulated by substrate availability and cellular metabolism. LuxS synthesizes AI-2, after which AI-2 accumulates extracellularly. AI-2 is imported by LsrACDB and phosphorylated by the kinase LsrK, which sequesters it within the cell. The phosphorylated AI-2 relaxes LsrR-mediated repression of the *lsr* operon, allowing transcription of *lsr* genes and accelerated AI-2 uptake. Several studies suggest that the bidirectional *lsr* operon, in addition to being regulated by LsrK and LsrR, is also subject to CCR. For example, activation of the *lsr* promoter does not occur in the presence of glucose (Wang et al., 2005) or glycerol (Xavier and Bassler, 2005) and requires the global regulators cAMP and CRP (Wang et al., 2005). Moreover, cAMP–Crp was shown to induce the *lsr* AI-2 uptake system gene by binding to its promoter (Xavier and Bassler, 2005). In addition, cAMP–Crp stimulates the production of the Hfq-binding sRNA CyaR, which can bind to and destabilize luxS mRNA, thereby reducing LuxS and AI-2 levels (De Lay and Gottesman, 2009). Ha et al. (2018) reported that HPr of *E. coli* co-purifies with LsrK. LsrK activity is inhibited when bound to HPr, revealing novel links between QS activity and sugar metabolism. A role for cAMP–Crp in the regulation of QS genes was also identified in the phytopathogen *Dickeya dadantii*, which contains the QS signal generator (*expI*) and the response regulator (expR). ExpR activates virulence genes in response to ExpI-made N-acyl-homoserine lactone (AHL) (Nasser et al., 1998; Reverchon et al., 1998). *In vivo* and *in vitro* studies have revealed that CRP functions as an activator of *expR* expression but as a repressor of *expI* transcription. This could explain the observation that the production of AHL decreases after quorum has been reached and when bacteria enter the stationary phase (Nasser et al., 1998). cAMP–Crp appears to be a modulator of QS gene expression, causing an increase in AHL production when less-preferred substrates are present. A regulatory effect of catabolic repression on QS signaling has been extensively studied in human-pathogenic bacteria such as *P. aeruginosa* PAO1, demonstrating a substantial impact of the Crc protein on QS-related social behavior, such as synthesis of virulence factors, biofilm formation, and fitness (Linares et al., 2010; Sonnleitner et al., 2012; Zhang et al., 2013). We hypothesize that the same could apply to rhizobacteria and that the dynamic nutritional environment of the host may have an impact via CCR on the cross talk between rhizosphere processes such as plant colonization and infection.

Catabolic repression-mediated control of virulence in plant pathogenic bacteria

In many pathogenic microorganisms, the CCR mechanism is crucial for virulence-gene expression and thus pathogenicity (Table 1). It should be noted that the primary aim of pathogenic bacteria is to access nutrients rather than to damage the host, and the expression of virulence genes is mostly linked to the nutrient supply of the bacteria (Görke and Stülke, 2008). For most pathogens, penetration through plant cell walls is of major importance for the invasion of host tissue and the acquisition of nutrients (Collmer and Keen, 1986). As a plant pathogen, *Dickeya* is capable of catabolizing a wide range of plant oligosaccharides and glycosides. The pectate lyases secreted by the bacterium provoke general disorganization of the plant cell wall and also release oligosaccharides, which are used as carbon sources by various *Dickeya* strains. Specific and global regulators serve to tailor pectate lyase (*pel*) gene expression to available substrates. Among them, the CAMP-CRP system is the best characterized (Nasser et al., 1997; Reverchon and Nasser, 2013). A *crp* mutation has serious consequences for the virulence of *D. dadantii*, in that it strongly decreases pectate lyase production (Reverchon et al., 1997). The *D. dadantii crp* mutant retains the ability to grow only on glucose, fructose, or sucrose, three efficiently metabolized carbon sources that are highly abundant in plant tissues (Hugouvieux-Cotte-Pattat and Charaoui-Boukerzaza, 2009). Thus, Crp plays a crucial role in the pathogenesis of *D. dadantii* by tuning the expression of virulence genes to the nutrient conditions encountered during plant infection.

The Crc/CrcZX system of *P. syringae* DC3000 is involved in the regulation of the type III protein secretion system (T3SS) encoded by *hpr* genes that deliver effectors into plant cells (Filiatrault et al., 2013). Indeed, sugars such as glucose, sucrose, and fructose are known to be inducers of the *P. syringae* T3SS genes, whereas intermediate tricarboxylic acids can suppress the T3SS *in vitro* through catabolite repression. The fructose and citrate utilization pathways used by *P. syringae* are upregulated when cells are exposed to tomato apoplast extracts (Rico and Preston, 2008). Filiatrault

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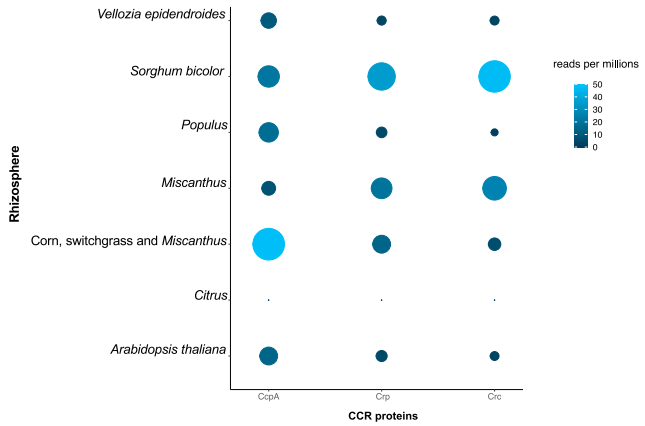


Figure 2. Carbon catabolite repression (CCR) gene expression in different plant rhizospheres.

The level of gene expression is quantified as the number of hits per 10^6 reads. Target genes are *crc* from *Pseudomonas fluorescens* F113, *ccpA* from *Bacillus subtilis*, and *crp* from *E. coli.* The rhizosphere metatranscriptomics bioproject accession numbers are, for *Vellozia epidendroides*, PRJNA441428; *Sorghum bicolor*, PRJNA406786; *Populus*, PRJNA375667; *Miscanthus*, PRJNA337035; corn, switchgrass, and *Miscanthus*, PRJNA365487; and *Arabidopsis thaliana*, PRJNA366978, PRJNA366977, and PRJNA336798.

et al. (2013) hypothesized that when the T3SS is active, expression of CrcZ and CrcX sRNAs may promote the utilization of carbon sources that are abundant in the plant apoplast. Moreover, Chakravarthy et al. (2017) provided evidence for a clear link between Crc and virulence in *P. syringae* DC3000. In this work, a Δ*crc* mutant caused fewer disease symptoms compared with the wild type in tomato and *Arabidopsis thaliana*. In addition, the *crc*-deficient mutant showed a delayed hypersensitive response when used to infiltrate *Nicotiana benthamiana* and tobacco. Taken together, these results demonstrated the direct and indirect roles played by Crc in nutrient acquisition and virulence-related traits.

CATABOLITE REPRESSION: EXPLORATION OF PREVALENT MECHANISMS IN BACTERIAL GENOMES

The CCR system has been described in different model strains, but information on its distribution among microbial genomes is still limited (Warner and Lolkema, 2003; Filiatrault et al., 2013). Here, we investigated the presence of CCR systems in bacteria through BLAST analysis in two genomic databases (NCBI and MAGE) using different CCR models that are genetically well characterized (see the supplemental information). We focused on bacteria in which CCR master genes are well known. The presence and conservation of those master genes allowed us to assess their distribution among various available bacterial genomes. It is worth noting that such analyses are dependent on the availability as well as the quality of the sequenced genomes. Therefore, it can be assumed that future studies will unravel the presence of CCR in additional bacterial genera and species.

Distribution of CCR among *Pseudomonas* and *Azotobacter* species

When assessing the CCR system in pseudomonads, we used several master genes that have been described as essential (Figure 2). We used the catabolic repression protein Crc, the global transcriptional regulator Hfq, the different sRNAs (CrcZ, CrcY, and CrcX), and the TCS CbrAB. Furthermore, we selected species with conserved homologous proteins (>40% identity in more than 80% of the aligned sequence, see supplemental information), synteny in the *cbrAB* genomic region, and potential involvement in plant–bacteria interactions, with the exception of *Pseudomonas oleovorans* T9AD, which was isolated from a marine environment. One or several genes in the CCR system have been functionally characterized in several *Pseudomonas* species, encompassing *P. putida*, *P. fluorescens*, *P. syringae*, and *P. aeruginosa* (Bharwad and Rajkumar, 2019). Our assessment indicated that the query proteins are widely distributed in this genus, suggesting their potential role in CCR (Table 2). Even though Hfq has a crucial function in CCR in *Pseudomonas*, its multiple functions in pleiotropic post-transcriptional regulation (Brennan and Link, 2007) may explain its high conservation among bacterial taxa (data not shown) and led us to not use it for the implemented screening. Thus, the genomic distribution of the CCR system was assessed using CbrAB, Crc, and sRNA (CrcX/Y/Z) sequences and showed high conservation only in pseudomonads (Table 2). Among the 26 species included here, genes from 5 had previously been described genetically as master genes and sRNAs involved in the CCR (Hester et al., 2000; Filiatrault et al., 2013; Liu et al., 2017; Quiroz-Rocha et al., 2017).

Azotobacter species, for example, *A. vinelandii*, also harbored the same, highly conserved CCR system found in *Pseudomonas* species (Table 2). Interestingly, it is known that this conservation is sustained, as the Crc-Hfq proteins from *A. vinelandii* and *P. putida* are functionally interchangeable (Quiroz-Rocha et al., 2017). In addition to these targeted proteins, CCR is mediated by sRNAs that antagonize the effect of Crc/Hfq proteins (Filiatrault et al., 2013). CrcX, CrcY, and CrcZ are the three sRNAs described to date. The CrcZ, CrcZ/CrcY, and CrcZ/CrcX sRNAs have been described in *P. aeruginosa* PAO1, *P. putida*, and *P. syringae*, respectively (Sonnleitner et al., 2009; Moreno et al., 2012; Filiatrault et al., 2013). Here, we combined BLAST and synteny analyses to identify and affiliate the best assignation of each sRNA (Table 2 and Supplemental Table 3). A more variable distribution of these sRNAs was observed among the *Pseudomonas* groups (Table 2). Three groups with or without CrcX/CrcY can be distinguished (Table 2). In the first group, which includes *P. syringae*, all three sRNAs are present. This suggests that in strains from the first group, all three sRNAs seem to be important and may work in concert, as suggested by Moreno et al. (2012). By contrast, strains in the second group, represented by *P. putida*, *P. fluorescens*, and *A. vinelandii*, harbored CrcZ/CrcY or CrcZ/CrcX sRNAs (Table 2). In *A. vinelandii*, both sRNAs (CrcZ/CrcY) play a key role in CCR (Martínez-Valenzuela et al., 2018), but this remains to be confirmed in the other species (Table 2). In the last group presented in Table 2, only CrcZ is present and seems to act solely as a unique sRNA in CCR, as demonstrated in

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Strain[a]	Ref_Seq	Habitat	Crc		CbrA		CbrB		*crcZ*		*crcY*		*crcX*	
			Cov (Id)	E-val	Cov (Id)	E-val	Cov (Id)	E-val	Cov (Id)	E-val	Cov (Id)	E-val	Cov (Id)	E-val
P. syringae pv. tomato DC3000[a,b]	NC_004578.1	Phytopathogen	100 (86)	0	99 (80)	0	99 (81)	0	94 (76)	0	31 (84)	3 e−22	100 (100)[b]	0
P. amygdali pv. *lachrymans* NM002	NZ_CP042804.1	Phytopathogen	100 (86)	0	99 (80)	0	99 (81)	0	94 (76)	0	100 (74)	5 e−30	100 (95)	0
P. cerasi isolate PL963	NZ_LT963395.1	Phytopathogen	100 (86)	0	99 (80)	0	99 (82)	0	96 (76)	0	100 (75)	2 e−38	100 (93)	0
P. coronafaciens pv. oryzae str. 1_6	NZ_CP046035.1	Phytopathogen	100 (87)	0	99 (79)	0	99 (82)	0	95 (77)	0	70 (78)	4 e−36	100 (90)	0
P. asturiensis CC1524	NZ_CP047265.1	Phytopathogen	100 (86)	0	99 (80)	0	99 (84)	0	94 (77)	0	100 (76)	7 e−39	98 (87)	0
P. putida KT2440[a,b]	NP_747393.1	Soil bacteria	100 (86)	0	99 (81)	0	99 (84)	0	60 (78)	5–35	100 (100)[b]	0	No	–
P. avellanae R2leaf	NZ_CP026562.1	Phytopathogen	100 (86)	6 e−174	99 (79)	0	99 (82)		92 (76)	0	No	–	100 (99)	0
P. viridiflava TA043	GCA_000452485.1	Phytopathogen	100 (87)	1 e−171	99 (81)	0	99 (83)	0	92 (76)	6 e−45	No	–	99 (87)	3 e−112
P. fragi A22	GCA_000250595.1	Stored chilled meats	100 (87)	8 e−173	99 (81)	0	99 (82)	0	92 (75)	1 e−36	90 (74)	4 e−29	No	–
P. extremaustralis 14-3	GCA_000242115.2	Highly stress-resistant Antarctic bacterium	100 (89)	6 e−175	99 (82)	0	100 (84)	0	93 (80)	3 e−68	90 (76)	1 e−43	No	–
P. brassicacearum NFM421	NC_015379.1	Soil bacteria PGPR	100 (88)	9 e−177	99 (81)	0	99 (83)		90 (76)	0	90 (77)	0	No	–
P. fluorescens F113[a]	NC_016830.1	Soil-denitrifying bacteria	100 (88)	2 e−173	99 (82)	0	100 (84)	0	90 (78)	2 e−54	90 (78)	5 e−53	No	–
P. protegens CHA0	NC_021237.1	Soil bacteria PGPR	100 (89)	4 e−175	99 (83)	0	99 (85)	0	90 (78)	2 e−62	93 (75)	4 e−57	No	–
P. chlororaphis ATCC 17415	NZ_CP027714.1	Soil PGPR bacteria	100 (89)	6 e−175	99 (82)	0	100 (84)	0	90 (78)	1 e−56	94 (74)	2 e−31	No	–
P. plecoglossicida NyZ12	NZ_CP010359.1	Pathogen of fish	100 (86)	2 e−170	99 (82)	0	99 (84)	0	60 (78)	1 e−32	100 (97)	0	No	–
P. entomophila L48	NC_008027.1	Soil bacteria and entomopathogen	100 (85)	7 e−168	99 (82)	0	99 (84)	0	95 (73)	4 e−27	97 (89)	3 e−125	No	–

Table 2. Similarity of CCR systems between *Pseudomonas* and *Azotobacter* strains

(Continued on next page)

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Strain[a]	Ref_Seq	Habitat	Crc		CbrA		CbrB		*crcZ*		*crcY*		*crcX*	
			Cov (Id)	E-val	Cov (Id)	E-val	Cov (Id)	E-val	Cov (Id)	E-val	Cov (Id)	E-val	Cov (Id)	E-val
Azotobacter vinelandii DJ[a]	NC_012560.1	Soil diazobacteria	100 (88)	1 e−176	98 (77)	0	99 (80)	0	90 (72)	2 e−42	94 (73)	1 e−46	No	–
Azotobacter chroococcum NCIMB 8003	NZ_CP010415.1	Soil diazobacteria	99 (86)	3 e−175	99 (78)	0	100 (81)	0	90 (71)	3 e−35	93 (72)	6 e−38	No	–
Azotobacter salinestris strain KACC 13899[c]	CP037918.1	Soil diazobacteria	99 (84)	0	99 (80)	0	98 (83)	0	ND	ND	ND	ND	ND	ND
P. aeruginosa PAO1[b]	NC_002516_2	Plant, animal, and human pathogen	100 (100)[b]	0	100 (100)[b]	0	100 (100)[b]	0	100 (100)[b]	0	No	–	No	–
P. balearica DSM6083	NZ_CP007511.1	Bioremediation (naphthalene)	100 (88)	5 e−176	99 (78)	0	99 (83)	0	27 (86)	1 e−27	No	–	No	–
P. psychrotolerans L19	GCA_000236825.2	Copper resistance	100 (84)	5 e−169	99 (75)	0	100 (81)	0	91 (72)	4 e−45	No	–	No	–
P. stutzeri DSM4166	NC_017532.1	Soil bacteria	100 (88)	8 e−175	99 (79)	0	100 (84)	0	92 (76)	6 e−45	No	–	No	–
P. denitrificans ATCC 13867	NC_020829.1	Soil-denitrifying bacteria (produce vitamin B12)	100 (89)	2 e−177	98 (89)	0	99 (89)	0	92 (86)	8 e−109	No	–	No	–
P. alcaliphila JAB1	NZ_CP016162.1	Bioremediation (phenol)	100 (89)	2 e−175	99 (83)	0	99 (87)	0	93 (80)	6 e−70	No	–	No	–
P. oleovorans T9AD	NZ_LR130779.1	Marine environment	100 (89)	8 e−175	99 (83)	0	99 (87)	0	93 (80)	6 e−70	No	–	No	–
P. pseudoalcaligenes CECT5344	NZ_HG916826.1	Bioremediation (cyanide)	100 (89)	3 e−175	99 (83)	0	99 (87)	0	93 (80)	5 e−66	No	–	No	–

Table 2. *Continued*

Coverage (Cov) and percentage of identity (Id) are indicated for each BLAST analysis.

[a]Species are already genetically described in terms of functional CCR systems (Hester et al., 2000; Filiatrault et al., 2013; Liu et al., 2017; Quiroz-Rocha et al., 2017). Habitat, taxonomy, and other phenotype information, including pathogenicity, were obtained from the NCBI microbial genomes database (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi).

[b]Analyses (PSI-BlastP for proteins and BlastN for sRNA and coding genes; see supplemental information) were performed using the sequences Crc (PA5332), CbrB (PA4726), CbrA (PA4725), and *crcZ* (PA4726.11) from *P. aeruginosa* PAO1; *crcY* (PP_mr44) from *P. putida* KT2440; and *crcX* (PSPTO_5669) from *P. syringae* pv. tomato DC3000 against references from MicroScope (https://mage.genoscope.cns.fr/microscope/home/index.php) and the NCBI database (https://www.ncbi.nlm.nih.gov/genome). Only species with conserved CbrAB synteny were selected for further analysis. Synteny was determined using MicroScope and the *Pseudomonas* browser (https://beta.pseudomonas.com/blast/setnblast).

[c]The percentage of identity, coverage, and E-value (E-val) were obtained by blastN. No proteins were annotated in the genome and, consequently, we could not affiliate sRNA found by blastN in NCBI (position 1, 1786973/1787336; position 2, 2234862/2235192) to the references (*crcZ*, *crcY*, or *crcX*) and also could not include them in the phylogeny analysis. ND, not determined.

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P. aeruginosa PAO1 (Sonnleitner et al., 2009), but this requires further investigation for all other species from this group. Possession of a single sRNA reflects a lesser need for fine-tuned regulation of metabolism compared with other pseudomonads, as previously suggested (Moreno et al., 2012).

Interestingly, the synteny of most sRNAs was conserved, as they harbored the same flanking gene in the left and right positions (Supplemental Table 1). CrcY is a unique sRNA with less conserved synteny. Indeed, 7 of the 13 assessed strains did not conserve Acyl-CoA dehydrogenase in the proximal region of CrcY, based on the use of *P. syringae* DC3000 as a reference. However, several of them conserved the same flanking gene as *crcY* of *P. putida* by harboring an MgtC transporter. Interestingly, *A. vinelandii* and *Azotobacter chroococcum* are the only species that also did not show conserved synteny in the downstream position of *crcY*. Indeed, the hydromethylglutaryl-CoA lyase (*mvaB*) gene was not present in the *crcY* right flanking region but rather in another locus of the *Azotobacter* genome (Supplemental Table 1). In the latter species, the affiliation of this second sRNA to *crcY* is questionable; however, it was retrieved with a high percentage of identity. Future experiments tracing the evolution of sRNAs in *Pseudomonas* and *Azotobacter* could reveal whether vertical or horizontal gene transmission has occurred between these species. Interestingly, the major *Pseudomonas* species shown to harbor this CRC system are not yet studied, although they are present in various ecological niches (Table 2) and associated with beneficial as well as pathogenic isolates. This reinforces the fact that the CRC system is involved in both beneficial and deleterious interactions. Remarkably, the strains that possess all the sRNAs are all pathogenic (Table 2), emphasizing that sRNA redundancy is likely to be important for this type of interaction. Even though the sRNAs are interchangeable for bacterial growth, as was demonstrated for CrcY and CrcZ in *P. putida* (Moreno et al., 2012), they may have different biological functions and confer distinct advantages or disadvantages under specific environmental conditions (Liu et al., 2017). Taken together, our results call for further investigations into the occurrence and redundancy of sRNAs that regulate CCR in phytopathogenic or beneficial strains as a response to plant exudate composition. This could facilitate optimization of their effects by triggering ecological functions such as QS, biofilm formation, and antibiotic or virulence factor production.

In addition, certain strains have been identified that are known to degrade xenobiotic compounds in soils (e.g., *P. pseudoalcaligenes* and *P. balearica*) (Table 2). Typically, these compounds are used as complex carbon sources, and their CCR-dependent degradation may reduce their ecological impacts on the environment. A good understanding of the fine regulation of the assimilation of these complex compounds by CCR could provide an ecological perspective for improving the biological remediation or assimilation of these pollutants in soil.

Distribution of pseudomonad CCR in other bacterial groups

Bioinformatics analysis using the same *Pseudomonas*-derived CCR sequences and synteny (CbrAB, Crc, and sRNAs) revealed the presence of this system in 98 other bacterial genera in addition to *Pseudomonas* and *Azobacter* (Supplemental Table 2). However, the percentage of sequence identity was substantially lower (40%–84%). Even though these proteins were annotated as homologous to those found in the pseudomonads group, functional experimentation must be performed to confirm their involvement in CCR. Interestingly, the distribution of Crc and CbrAB was found to be more restricted to γ-proteobacteria covering 11 different orders; there were only two exceptions, belonging to Firmicutes and to β-proteobacteria (Supplemental Table 2). Among all analyzed species, we did not find *cbrAB* in the genomes of *Acinetobacter baylyi*, *Acinetobacter baumanii*, or *Vibrio cholerae,* even though the roles of Crc and Hfq have been previously investigated, but the direct link to catabolic repression has not been demonstrated (Zimmermann et al., 2009; Vincent et al., 2012; Kuo et al., 2017). Notably, NtrBC, a TCS that plays an important role in N source utilization, has also been described as playing a pivotal role in the C/N balance in concert with the CbrAB system (Li and Lu, 2007). In addition, NtrBC was widely distributed in Proteobacteria, including the genomes of *A. baylyi*, *A. baumanii*, and *V. cholerae* (data not shown). Thus, in Proteobacteria that do not conserve CbrAB, it would be interesting to investigate the role of NtrBC with regard to the CCR system, as has already been characterized in *P. fluorescens* SBW25 for histidine utilization (Naren and Zhang, 2021). For the main species harboring the homologous CbrAB and Crc proteins (Supplemental Table 1), a blastN for all sRNAs (CrcZ/X/Y) was performed but provided no results. This result suggests the involvement of other divergent sRNAs, the activity of the CCR system in an sRNA-independent manner, or inefficiency of the CCR system owing to a lack of genetic regulation. Three species (*Paucimonas lemoignei*, *Priestia aryabhattai*, and *Stenotrophomonas rhizophila*) from different orders are exceptions, as they harbor one or two sRNAs in their genomes (Supplemental Table 1), suggesting possible horizontal gene transmission. Regarding their habitat, and unlike the pseudomonads groups (Table 2), the identified bacteria are found mostly in aquatic habitats (Supplemental Table 2), which offers a novel view of the implications of the CCR system for this less-investigated ecosystem. Certain bacteria that are capable of fixing N (e.g., *Teredinibacter turnerae*, *Azomonas agilis*) may rely on the CCR system, as has been found for telluric bacteria (e.g., *Azotobacter*). This suggests that CCR systems are not limited to carbon recycling, as observed in *Pseudomonas* species, but may also be involved in N assimilation. In a broader context, further study of these bacterial models, including aquatic strains, could provide evidence for other environmentally relevant functions regulated by CCR.

Distribution of catabolic repression mechanisms known from *E. coli*

To investigate the distribution of the *E. coli* CCR system across various bacterial taxa, we looked for high conservation (>40% identity in >80% of the aligned sequence) of the three master proteins in this system: Crp, CyaA, and the glucose-specific component EIIA. We documented the wide dispersal of this CCR system (Supplemental Table 3), and 54 different genera were shown to possess these three conserved proteins (Supplemental

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Class	Strain	Ref_Seq	Habitat	Coverage (identity)[a]			Database
CCR system in Enterobacteriaceae				**Crp**	**CyA**	**EIIA**	
δ-proteobacteria	*Escherichia coli* K12	ECK.1	Laboratory strain (reference)	100 (100)	100 (100)	100 (100)	MAGE
	Atlantibacter subterranea AS_373	RHXB01.1	Contaminated subsurface sediment	100 (99)	100 (92)	100 (92)	MAGE
	Citrobacter koseri ATCC BAA-895	NC_009792.1	Ubiquitous in soil and water (nitrogen fixing)	100 (99)	100 (96)	100 (98)	MAGE
	Dickeya dadantii 3937*	NC_014500.1	Phytopathogen	100 (99)	100 (83)	100 (95)	MAGE
	Erwinia carotovora subsp. *atroseptica* SCRI1043 (or *Pectobacterium carotovorum*)[b]	NC_004547.2	Phytopathogen	100 (99)	98 (83)	100 (95)	MAGE
	Pantoea agglomerans IG1	NZ_BAEF.1	Plant symbiont (N fixing)	100 (99)	99 (83)	100 (87)	MAGE
	Brenneria nigrifluens DSM 30175	NZ_CP034036.1	Phytopathogen	99 (99)	83 (97)	100 (94)	NCBI
	Phaseolibacter flectens	NZ_JAEE01000001.1	Phytopathogen	99 (94)	97 (67)	100 (73)	NCBI
	Tolumonas auensis	NC_012691.1	Toluene producer	99 (90)	96 (52)	100 (83)	NCBI
	Zobellella maritima	NZ_QCZE01000001.1	Sediments (degrades polycyclic aromatic hydrocarbons)	99 (88)	98 (50)	100 (78)	NCBI
	Jejubacter calystegiae strain KSNA2	NZ_CP040428.1	Stem tissue of *Calystegia soldanella* plant	99 (99)	100 (88)	100 (89)	NCBI
	Lelliottia amnigena strain NCTC12124	NZ_CP077331.1	Soil	99 (98)	100 (92)	100 (99)	NCBI
	Kosakonia arachidis strain KACC 18508	NZ_CP045300.1	Soil	99 (100)	100 (93)	100 (98)	NCBI
β-proteobacteria	*Thauera selenatis* AX ATCC 55363	NZ_CACR.1	Sediments	100 (99)	100 (94)	100 (98)	MAGE
CCR system in Firmicutes				**CcpA**	**HprK**	**HPr**	
Firmicutes	*Bacillus subtilis* 168	BSU.1	Laboratory strain	100 (100)	100 (100)	100 (100)	MAGE
	Bacillus amyloliquefaciens Bs006[b]	LJAU.1	PGPR bacteria (*Physalis peruviana* roots)	100 (95)	100 (96)	100 (100)	MAGE
	Peribacillus acanthi	NZ_QBBX01000001.1	Rhizobacteria (*Acanthus ilicifolius*)	100 (75)	99 (78)	100 (70)	NCBI
	Pullulanibacillus pueri	NZ_BMFV01000001.1	Plant (tea)	98 (73)	96 (65)	100 (61)	NCBI
	Pueribacillus theae	NZ_QCZG01000001.1	Plant (tea)	98 (71)	99 (71)	97 (44)	NCBI
	Ammoniphilus oxalaticus	NZ_MCHY01000001.1	Rhizosphere (*Rumex acetosa*)	98 (64)	97 (60)	95 (46)	NCBI
	Fontibacillus phaseoli	NZ_QPJW01000001.1	Plant nodules (*Phaseolus vulgaris*)	98 (63)	97 (68)	97 (55)	NCBI
	Saccharibacillus sacchari DSM 19268	JFBU01.1	Endophyte (*Saccharum officinarum*)	98 (63)	98 (65)	98 (54)	MAGE

Table 3. CCR system comparison in *Escherichia coli* and *Bacillus* species.

(Continued on next page)

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Class	Strain	Ref_Seq	Habitat	Coverage (identity)[a]			Database
	Paenibacillus polymyxa ATCC 15970	CP011420.1	PGPR bacterium (N fixing)	98 (62)	99 (65)	99 (54)	MAGE
	Bhargavaea beijingensis	NZ_FNAR01000001.1	Root of a ginseng plant	98 (59)	96 (64)	97 (48)	NCBI
	Atopococcus tabaci	NZ_AUCD00000000.1	Plant (tobacco)	99 (58)	96 (52)	100 (63)	NCBI

Table 3. *Continued*

PSI-BlastP analyses were conducted with the sequences Crp (ECK3345), CyaA (ECK3800), and PTS (ECK2412) proteins from *E. coli* (strain K12) or CcpA (BSU29740), HprK (BSU35000), and HpR (BSU13900) proteins from *Bacillus subtilis* (strain 168) using MicroScope and NCBI genome databases. The *E. coli* CCR system was found in different Proteobacteria, and the *Bacillus* CCR system was present in members of Firmicutes. This list was extracted for Supplemental Tables 3 and 4 by selecting only telluric bacteria and/or bacteria associated with plants (endophytic, epiphytic, or rhizobacteria). Habitat, taxonomy, and other phenotype information, including pathogenicity, were obtained from the NCBI microbial genomes database (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi). Information concerning strains used is provided according to the genome browser.

[a]All E-values are <10 E^{-10} and are included in Supplemental Tables 3 and 4.

[b]Species have already been described as harboring functional CCR systems (Tsuyumu, 1979; Reverchon et al., 1991; Chen et al., 2020).

Table 3). Interestingly, most bacteria were assigned to γ-proteobacteria, with only one exception (*Thauera selenatis*, assigned to β-proteobacteria) (Supplemental Table 3). Moreover, *Pseudomonas* (γ-proteobacteria) strains showed the presence of a conserved Crp protein in their genomes, but the other two proteins (CyaA/EIIA) were either not conserved or absent. The presence of Crp in *P. putida* has been reported previously, but the growth of a *crp* mutant was not affected by the carbon compounds tested and was impaired only by the use of various dipeptides as a nitrogen source (Milanesio et al., 2011). These results provided clear evidence that CCR is not Crp dependent in *Pseudomonas*.

Overall, we observed a high occurrence of this CCR system among Proteobacteria from diverse habitats. It is worth noting that a large proportion of the detected species are associated with animals or insects as pathogens, symbionts, or endophytes. CCR is poorly documented in these host-associated microbes. Among the detected species, some have been reported to degrade pollutants or fix nitrogen (Table 3), supporting previous suggestions related to the assimilation of complex carbon compounds and N by bacterial communities through fine regulation of CCR. Other detected species have been described as associated with plants, either as symbionts or pathogens, demonstrating once again the role of CCR in crucial ecological functions involved in plant–bacteria interactions.

Distribution of CCR mechanisms from *Bacillus* across other bacterial taxa

To investigate the distribution of CCR mechanisms known from *Bacillus* in other bacterial taxa, we searched for high conservation (>40% identity in >80% of the aligned sequence) of three master proteins: the phosphocarrier HPr, its kinase/phosphorylase HPr-K/P, and the catabolic control protein CcpA. This exploration resulted in the identification of 168 different genera (Supplemental Table 4) that almost all belong to the class Firmicutes. The two exceptions were assigned to *Haloplasma contractile* and *Mycobacteroides abscessus* subsp. *abscessus*, which showed variation in identity scores (46%–80%) for the query proteins. Similarly, *Rhizobiales* species are known to harbor the HPr-K protein, but our analysis showed high divergence from the well-characterized proteins involved in the *Bacillus* CCR system (data not shown). Furthermore, *Rhizobiales* species possessed a gene encoding the glucose-specific component EIIA described in Proteobacteria, but this protein was also markedly divergent from the one found in *E. coli* (identity <40%). The role of these proteins in the CCR of *Rhizobia* has already been demonstrated (Table 1). Remarkably, *Rhizobiales* combine genes encoding CCR from Proteobacteria and Firmicutes in their genomes, but their high divergence suggests a complex evolutionary history.

In Firmicutes, this system also showed a broad range of divergence (from 40% to 100% identity), regardless of the targeted proteins, suggesting a more relaxed pressure for substitutions in these proteins if they are functional (Supplemental Table 4). Bacteria possessing this CCR system were found to originate from various habitats, including soil and seawater. Several bacteria have been isolated from aliments and/or used for industrial applications such as fermentation to produce metabolites or degrade organic components. Thus, adjustments to their CCR systems could provide a viable strategy to optimize biomass production for industrial purposes. In terms of plant–bacteria associations, we identified 10 species isolated from plant tissues or from the rhizosphere (Table 3). Interestingly, all plant pathogens were less present, while human (e.g., *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae*) and animal/insect (e.g., *Melissococcus plutonius*) pathogens were more represented (Supplemental Table 4). This could be explained by the fact that Firmicutes contain fewer representative phytopathogens compared with other classes. They more commonly include PGPR bacteria, such as *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*. Some of them are commonly used as biological control agents against phytopathogens in agriculture (e.g., *B. subtilis*).

In addition, several phylogenetic trees were constructed for each CCR system by including global master proteins (Crc, Crp, or CcpA; Supplemental Figures 1–3) in order to safely assign relationships, as well as reciprocal best hits. Globally, the trees confirmed the phylogenetic link between master proteins found

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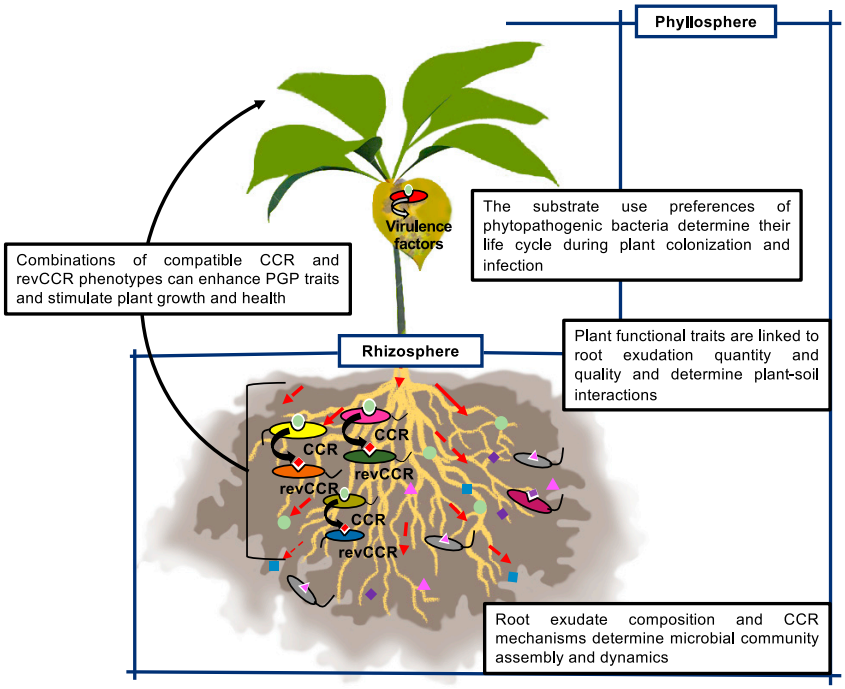


Figure 3. Carbon catabolite repression (CCR) mechanisms involved in plant–microbe interactions.

A schematic overview of how plant root exudates can regulate microbiome assembly, diversity, and function via CCR mechanisms. The quality and quantity of root exudates are linked to plant functional traits. Microorganisms that use CCR and revCCR coexist in the rhizosphere of plants. Those using revCCR may consume the by-products of other members of the plant microbiota that use CCR metabolism. Primary metabolites secreted by plants and present in vascular tissues may regulate the phytopathogenic and beneficial properties of bacteria via CCR.

in all strains, except for *Haemophilus influenzae* for Crp, as well as Enterobacteriaceae and *Haloplasma contractile* for CcpA. Sequences from Firmicutes, which generate long branches, appear to have a more complex evolutionary history, which will require deeper investigation in upcoming studies.

CATABOLITE REPRESSION: IDENTIFICATION OF MECHANISMS EXPRESSED IN PLANT RHIZOSPHERES

In a targeted approach, we explored whether genes encoding catabolite repression are expressed in the plant rhizosphere. We used different available rhizosphere metatranscriptomic datasets for BLAST analyses. Three protein sequences from three model strains were selected as queries: Crc from *P. fluorescens* F113, CcpA from *B. subtilis* (strain 168), and Crp from *E. coli* (strain K12). In total, 10 rhizosphere metatranscriptomic bioprojects available at NCBI were included (Supplemental Table 5). The datasets were obtained from rhizosphere soil and/or roots of *Arabidopsis*, corn, switchgrass, maize, canola, sorghum, *Miscanthus*, *Populus*, and *Vellozia epidendroides*. The reads were assembled, filtered, de-replicated, and screened for chimeras in order to obtain high-quality reads. We then compared the sequence similarity of the reference protein sequences against these unique reads by Diamond BLAST analysis (at least 40% identity and 80% coverage). We found that genes involved in catabolic repression are expressed in the rhizospheres of *A. thaliana*, *V. epidendroides*, *Populus*, *Miscanthus*, corn, switchgrass, and sorghum, suggesting a potentially important role for this mechanism in the regulation of plant–bacteria interactions in the rhizosphere (Figure 2). Notably, *ccpA* showed greater expression than *crp and crc* in corn, switchgrass, and *Miscanthus*, whereas *crc* showed greater expression than *ccpA* and *crp* in *Sorghum bicolor*. Interestingly, bacteria using CCR and organic acid–mediated CCR coexist in the plant rhizosphere, as previously observed in medical environments, including chronic wounds and cystic fibrosis lungs (Orazi and O'Toole, 2017). Bacteria using revCCR may consume the by-products of CCR metabolism and thereby remove metabolites that could be a thermodynamic constraint on metabolism, as well as being inhibitory (Brileya et al., 2014). This positive feedback mechanism would enable consortia to increase biomass productivity (Park et al., 2020). For example, *P. fluorescens* F113 (organic acid–mediated CCR) and *B. subtilis* (CCR) are excellent rhizosphere colonizers through root biofilm formation and are often isolated together. Their synergistic interactions in plant roots are likely to improve plant colonization through more efficient resource acquisition and greater efficiency of converting resources into biomass. At the bacterial community level, the complementary properties of the two strategies (CCR and organic acid–mediated CCR) can mitigate direct competition for energy and nutrients and instead establish a cooperative division of labor in the rhizosphere. We therefore suggest that CCR is a dynamic process that is important for bacterial community assembly and hence for ecosystem function. Interpretation of global regulation processes based on consortia rather than single cultures is needed for a better understanding of the plant rhizosphere microbiota and can contribute to future applications in agriculture.

FUTURE CONSIDERATIONS

Plants adopt different strategies to interact with their environment for the acquisition or conservation of nutrients. Many nutrient-use strategies can be explained by plant functional traits (Violle et al., 2007). Plants with higher photosynthetic capacity and efficient N uptake are referred to as exploitative (or fast-growing) plant species, whereas plants with lower nutrient uptake and photosynthetic activity but higher levels of leaf and root dry mass are considered conservative (or slow-growing) plant species (Aerts and Chapin, 1999). These contrasting strategies influence the input and output of C resources. Guyonnet et al. (2017, 2018) explored the influence of plant nutrient-use strategies on the level and quality of root exudation. They found that exploitative plant species exude more C in general, as well as more

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diversified C, than conservative species. This exudation plays a crucial role in shaping rhizosphere communities. Different studies have demonstrated the effects of root exudation on the diversity and function of the plant microbiome (Bulgarelli et al., 2013; Guyonnet et al., 2018), termed the "rhizosphere effect." However, this effect has not, to date, been linked to CCR regulation. Until recently, no study had addressed the CCR regulatory effects of different root exudates in terms of the structure, diversity, assembly, and function of the microbiome. How does the nature of root exudates influence plant growth and health by shaping the diversity and function of the plant microbiota? How does the nature of primary metabolites contained within vascular tissues, such as xylem and apoplasts, regulate bacterial virulence? These questions could be addressed using *A. thaliana* mutants specifically altered in the exudation of sugars or organic acids. Analysis of the microbiome using metabarcoding approaches coupled with metatranscriptomics in isogenic mutants for a given sugar or organic acid could provide insight into the importance of CCR for rhizosphere functions. Approaches to accurately characterize the metabolite composition of root exudates under natural conditions, as done by Guyonnet et al. (2017), but coupled with metabarcoding and metatranscriptomics, are also needed for a better understanding of the plant's capacity to shape the diversity and functions of its microbiota (Figure 3).

The substrate use preferences of rhizobacteria and phytopathogenic bacteria, regulated by CCR, determine the life cycle of bacteria during root colonization and infection. Analysis of transcriptomes from *D. dadantii* in synthetic cultures after exposure to different carbon sources mimicking the environment encountered in the apoplastic space during plant infection suggests that during the first stage of infection (asymptomatic phase), in the presence of sucrose and polygalacturonate (a pectin derivative), *D. dadantii* uses sucrose as a C source, and the *pel* genes encoding pectate lyases, responsible for soft rot symptoms, are repressed during the exponential phase of growth. At the end of the exponential growth phase (symptomatic phase), the *pel* genes are activated by the cAMP-CRP complex, and the bacteria begin to use polygalacturonate as a carbon source (Jiang et al., 2016). Analysis of the fate of strains mutated in genes encoding the CCR mechanism under natural conditions in the rhizosphere will be relevant to understanding trait regulation of bacteria during their life cycle. Are these mutants competitive, and do their beneficial or pathogenic functions continue to be expressed? Transcriptomic and metabolomic analysis of CCR mutants *in planta* is needed to respond to these questions. Beyond bacteria, these questions could also be applied to the whole plant microbiome, as its prokaryotic and eukaryotic members are constantly interacting with one another, resulting in specific adaptations.

Previous studies that have focused on improving plant growth and health have also contrasted single strains of rhizobacteria with different strain combinations as inoculum. Some of them demonstrated a significant improvement in plant growth and health, whereas others showed no effect (Oleńska et al., 2020). For example, Ansari and Ahmad (2019) observed a significant enhancement of vegetative growth and photosynthetic parameters of wheat seedlings growing in natural soil after co-inoculation with *P. fluorescens* strain FAP2 and *Bacillus licheniformis* B642 compared with single inoculation of each strain alone. The authors demonstrated a positive interaction between the two strains and showed that both harbored multiple plant growth-promoting traits, such as the production of auxin or siderophores and the solubilization of phosphate. The positive interaction between these bacteria may be explained by their substrate use preferences. Indeed, *Bacillus* species are known to prefer sugars; they primarily use glucose exuded by wheat seedlings, maximizing their growth rate and the production of secondary metabolites, which affects plant performance. By contrast, *Pseudomonas* prefers organic acids and can use acetate and other by-products produced by *Bacillus* to grow and produce secondary metabolites. It can therefore be assumed that combinations of compatible CCR and organic acid–mediated CCR phenotypes can enhance plant growth-promoting traits and stimulate plant growth and health by enabling cooperation via substrate allocation provided by the host plant. Future design of highly efficient rhizobacterial consortia will rely on a detailed understanding of their CCR mechanisms to ensure compatibility with plant root exudates and fully harness their potential for plant health and growth promotion.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.

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AUTHOR CONTRIBUTIONS

Conceptualization, F.Z.H. and H.B.; methodology, T.F., D.A., H.B., and F.Z.H.; investigation, T.F., D.A., H.B., and F.Z.H.; writing – original draft, T.F., H.B., and F.Z.H.; writing – review & editing, T.F., W.A., T.C., W.N., S.R., H.B., and F.Z.H.; funding acquisition, F.Z.H.; resources, H.B. and F.Z.H.; supervision, F.Z.H.

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