One-Component Systems that Regulate the Expression of Degradation Pathways for Aromatic Compounds

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Contents

Abstract

The expression of pathways for the catabolism of aromatic compounds is energetically expensive, and aromatic compounds are generally toxic even to bacteria that can use them as growth substrates. Hence, complex regulatory circuits that control the expression of the degradation pathways have evolved. Transcriptional

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regulation appears to be the most common mechanism for control of gene expression. Effector-specific transcriptional regulation of aromatic catabolic pathways depends on the performance of a specific regulator acting on a specific promoter and responding to a specific effector signal. One-component regulatory systems combine within the same cytosolic protein the effector-binding input domain and a DNA-binding output domain. A great variety of one-component regulatory systems can be classified within different families of prokaryotic transcriptional regulators revealing a wide diversity in their evolutionary origins and showing that a regulatory issue, i.e., having an operon induced in the presence of a given aromatic compound, can be solved through different types of regulators and mechanisms of transcriptional control in different bacteria. The effector-specific regulation can be tightly fine-tuned by the action of certain modulators and is, in turn, under control of overimposed mechanisms that connect the metabolic and energetic status of the cell to the activity of the individual catabolic clusters, leading to complex regulatory networks. Elucidating such regulatory networks will pave the way for a better understanding of the regulatory intricacies that control microbial biodegradation of aromatic compounds, which are key issues that should be taken into account for the rational design of more efficient recombinant biodegraders, bacterial biosensors, and biocatalysts for modern green chemistry.

1 Introduction

Aromatic compounds are the second most widely distributed class of organic compounds in nature (Díaz et al. 2013). Although some of these compounds are recalcitrant or toxic for the vast majority of the microorganisms, bacteria usually have evolved biochemical and genetic information that allow them to use the aromatic compounds as a sole carbon and energy sources (Lovley 2003). The production of the multiple enzymes of pathways for the catabolism of aromatic compounds is energetically expensive, and aromatic compounds are generally toxic even to bacteria that can use them as growth substrates. Hence, complex regulatory circuits that control the expression of the degradation pathways have evolved (Lovley 2003; Cases and de Lorenzo 2005; Carmona et al. 2008; Díaz et al. 2013). Although regulation can be carried out at different levels (transcription, translation, posttranslation), transcriptional regulation appears to be the most common mechanism for control of gene expression. Transcriptional regulation of aromatic catabolic pathways is not just dependent on the performance of a specific regulator acting on a specific promoter and responding to a specific environmental signal (effector-specific transcriptional regulation) but is also dependent on overimposed mechanisms that connect the metabolic and energetic status of the cell to the activity of the individual catabolic clusters (Fig. 1) (Díaz and Prieto 2000; Tropel and van der Meer 2004; Carmona et al. 2008; Díaz et al. 2013). In this chapter, we will review the effector-specific transcriptional regulation involved in the catabolism of aromatic compounds.

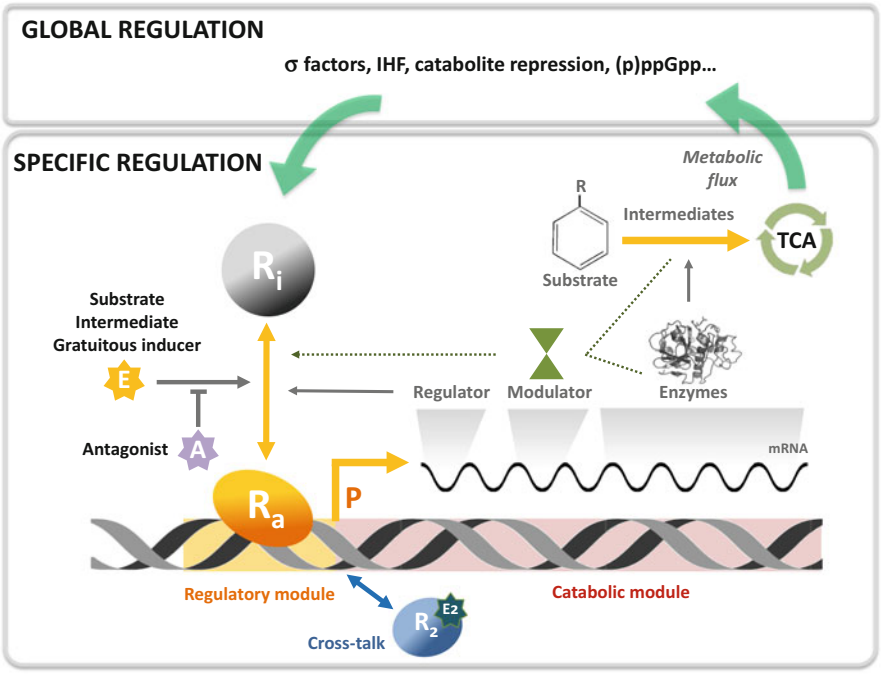

Fig. 1 Scheme of the regulatory network that controls the expression of genes responsible for the catabolism of aromatic compounds. The effector-specific regulator can be either in its inactive (Ri) or active and bound to DNA (Ra) forms. The interaction with the effector molecule (E) determines the transition between both states, leading to derepression (as in case of a repressor) or activation (as in case of an activator) of gene expression from the target promoter (P). Structural analogues of the effector molecules may act as antagonists (A) preventing the action of the former. Auxiliary regulators (modulator), that interact with the enzymatic machinery or some pathway intermediates, may control the activation of the main regulator modifying its final regulatory output. Additional regulatory loops based on cross-talk regulation of the target promoter by a different regulator (R2) and effector (E2) couple can exist. The specific regulation is generally subjected to a more global level of regulation dependent on the overall physiological state of the cell and that in turn responds to the final metabolic flux derived from the funneling of the cognate aromatic compound to the central metabolism (TCA)

2 Effector-Specific Transcriptional Regulators Involved in the Catabolism of Aromatic Compounds: General Features

Signal transduction in prokaryotes is conducted by two major regulatory systems, (i) one-component systems and (ii) two-component systems. Two-component regulatory systems function as a result of phosphotransfer between two key proteins, a sensor histidine kinase (input element) and a cytosolic response regulator (output element). One-component systems combine within the same cytosolic protein the

sensor input domain and a functional output domain. Small molecule-binding motifs constitute the majority of the input domains, and helix-turn-helix (HTH) DNA-binding motifs are the most common output domains (Ulrich et al. 2005). In this chapter, however, we will focus only on one-component transcriptional regulatory systems that, in the presence of the inducer molecule (effector), assure the production of the enzymes and transporters involved in the catabolism of aromatic compounds either by activating (activator) or derepressing (repressor) their cognate genes (Fig. 1).

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Regulators that show similar domain architectures might be responsible for different biological effects depending on the locations of their binding sites (operator regions) in the cognate promoters. Thus, the same regulator can activate some genes when it binds upstream of the RNA polymerase (RNAP)-binding sites while repressing others when it binds downstream of such RNAP-binding regions. The inducer molecule that activates the transcriptional regulator can be the pathway substrate and/or a pathway intermediate or product, or some structural analogues of the natural effector (gratuitous inducer) that may not themselves be a substrate for the corresponding catabolic pathway (Fig. 1). Some specific regulators have more than one effector-binding pockets, and the cognate effector molecules may have peculiar synergistic effects on transcriptional activation (Manso et al. 2009). On the contrary, efficient recognition of molecules (antagonists) that show structural similarity to the inducers (agonists) by certain transcriptional regulators leads to a lack of activation of the target promoter (Fig. 1), which may compromise an efficient degradation response when bacteria are exposed to complex mixtures of aromatic pollutants, some of which behave as agonists and other as antagonists (Silva-Jiménez et al. 2011). To prevent the gratuitous induction by non-metabolizable analogues or nonproductive intermediates, some regulatory proteins (modulators), e.g., ThnY and PaaY (García et al. 2011; Fernández et al. 2013; Ledesma-García et al. 2016), are coupled to the aromatic degradation enzymes in order to induce gene expression when there is an efficient catabolic flux in the cell (Fig. 1).

Although both transcriptional activators and repressors have been shown to regulate aromatic catabolic pathways, those pathways that use CoA-derived

aromatic compounds are mainly controlled by transcriptional repressors that recognize CoA-derived effector molecules (Sakamoto et al. 2011; Hirakawa et al. 2012; Valderrama et al. 2012; Juárez et al. 2015). This observation may reflect that repressors are generally preferred to control low-demand genes whose unspecific transcription can decrease the overall fitness of the cell by spending valuable resources, such as CoA and ATP, on futile processes (Sasson et al. 2012). In some cases, the transcription factors control a set of different functionally related metabolic clusters, e.g., the PhhR regulon that assures the homeostasis of aromatic amino acids in *Pseudomonas putida* (Herrera et al. 2010).

The acquisition of specificity for a new inducer in a transcriptional regulator requires a pre-existing regulator with a certain escape (responsiveness to non-legitimate effectors or regulatory noise), upon which new specificity can be built by several rounds of natural or artificial mutagenesis and selection. In fact, regulators of microbial pathways for recent compounds (e.g., aromatic xenobiotics) are not too specific for their substrates, which may reflect an ongoing evolution of as yet not entirely optimized regulation in response to unusual nutrients (Cases and de Lorenzo 2005). In *Burkholderia* sp. DNT, the regulation of the 2,4-dinitrotoluene (DNT) degradation pathway is in an earlier stage of evolution since the NtdR regulator still recognizes salicylate, an effector of its NagR-like ancestor, but does not respond to 2,4-DNT. That a useless but still active transcriptional factor operates along enzymes that have already evolved a new substrate specificity points to the fact that the emergence of novel catalytic activities precedes the setting of a specific regulatory device for their expression, not vice versa. This shades some light into the chicken-and-the-egg dilemma between regulators and enzymes that recognize the same compounds (de las Heras et al. 2011). The evolution of transcriptional regulators has also been assessed by in vitro experimental evolution/selection setups. For instance, the XylR regulator from *P. putida* was evolved first to an effector-promiscuous variant and then to a more specific regulator where the natural response to *m*-xylene was decreased and the nonnative acquired response to the synthetic 2,4-DNT was increased. The new XylR28 version may be used to develop more efficient 2,4-DNT responsive reporter systems to engineer whole-cell biosensors for explosives (de las Heras and de Lorenzo 2011). The promiscuity or specificity of inducer recognition might be also tuned in a regulatory network just by changing the promoter architecture and without requiring the evolution of new transcription factors with altered inducer specificity, e.g., the 3-methylbenzoate-dependent induction of the *ben* operon for benzoate degradation by the BenR regulator (Silva-Rocha and de Lorenzo 2012), or the participation of some global regulators in the activation of certain promoters, e.g., the ppGpp−/DksA-independent co-stimulation of the *dmpR* regulatory gene that controls phenol degradation (del Peso-Santos et al. 2011) in *P. putida*.

Transcriptional regulators may form regulatory cascades that involve the interplay between two or more proteins controlling a pathway for the degradation of aromatic compounds (Fig. 1). Although these circuits are mainly based on an activation strategy, e.g., the XylR/XylS (Silva-Rocha et al. 2011), AadR/HbaR (Egland and Harwood 2000), and PhcT/PhcR (Teramoto et al. 2001) regulatory

pairs, there are also examples of cross regulation between two transcriptional repressors that control subsequent steps in a catabolic pathway (Jiménez et al. 2011). These regulatory networks may help to maintain the catabolic pathways as an autonomous metabolic machinery that interacts only minimally with the central carbon consumption routes of the host cells (orthogonality), thus facilitating a quick spread of such degradation routes through the microbial population under suitable environmental pressure (Silva-Rocha et al. 2011).

Cross regulation between different aromatic catabolic pathways may assure a tight control of gene expression (Valderrama et al. 2012), prevent the expensive expression of funneling pathways that produce the cognate compound used as growth substrate (vertical regulation) (del Peso-Santos et al. 2006), or select for a specific pathway when mixtures of substrates that are feeding into different pathways are provided as carbon source (Bleichrodt et al. 2010). Usually there is a hierarchical use of aromatic compounds when bacteria grow in mixtures of these carbon sources in the environment, e.g., benzoate is usually a preferred carbon source over 4-hydroxybenzoate. Whereas the 4-hydroxybenzoate transport gene (*pcaK*) has been proposed as the main target of the repression in *Acinetobacter baylyi* (Brzostowicz et al. 2003) and *P. putida* (Cowles et al. 2000), the 4-hydroxybenzoate hydroxylase gene (*pobA*) is the key controlled element in *C. necator*, being benzoate itself the molecule mediating the repression through a possible interaction with the PobR regulator (Donoso et al. 2011; Pérez-Pantoja et al. 2015). Interestingly, the aromatic preference profile can change even between closely related strains (Jõesaar et al. 2010). Nevertheless, there are, of course, examples of simultaneous degradation of aromatic compound-containing mixtures, including synergistic interactions as those described in *Sagittula stellata* where increased growth rates were observed when cells were provided with benzoate/4-hydroxybenzoate mixtures compared to cells grown singly with an equimolar concentration of either substrate alone (Gulvik and Buchan 2013). Remarkably, the recent observation that there is cross regulation between aerobic and anaerobic degradation pathways could be an adaptive advantage for certain bacteria that thrive in changing oxygen environments (Valderrama et al. 2012).

Most promoters in an environmental context are regulated as part of complex circuits involving several global transcription factors (e.g., σ factors) (Fig. 1). In bacteria, transcription factors are usually present at a few copies per cell, which unavoidably leads to fluctuations in protein abundance and thus to cell heterogeneity in isogenic cell populations. This means that individual cells in the population, which may either exist in different growth phases and thus expressing different σ factors or in the same growth phase but expressing different levels of transcription factors, can activate transcription at the same promoter to different degrees. The existence of subpopulations that express differentially certain catabolic pathways can in turn favor the adaptation of the cell community to unpredictable environmental changes (Guantes et al. 2015).

One-component regulatory systems can be classified within different families of prokaryotic transcriptional regulators based on the sequence and structure of the DNA-binding motif of the output domains. Examples of regulators involved in

controlling the expression of aromatic catabolic pathways reveal a wide diversity in their evolutionary origins and show that a regulatory problem, i.e., having an operon induced in the presence of a given aromatic compound, can be solved through different types of regulators and mechanisms of transcriptional control in different bacteria (Table 1). Moreover, this global view confirms that the regulatory networks have an extraordinary degree of plasticity and adaptability, reinforcing the idea that catabolic and regulatory genes in aromatic degradation pathways have evolved independently (Cases and de Lorenzo 2005; Carmona et al. 2008).

Below, we briefly explain the general features of the different families of one-component transcriptional regulators controlling the catabolism of aromatic compounds, paying special attention to those examples that have been characterized in the last 10 years. Other reviews on various aspects of the regulation of aromatic degradation pathways have been previously published (Díaz and Prieto 2000; Tropel and van der Meer 2004; Carmona et al. 2008). Two-component transcriptional regulators are extensively discussed in a different chapter of this series.

3 Aromatic Compound Responsive Regulators of the LysR Family

The LysR family of transcriptional regulators (LTTRs) represents the largest family of bacterial transcription factors (Pareja et al. 2006; Maddocks and Oyston 2008), and family members regulate the expression of a wide variety of biological functions (Schell 1993; Tropel and van der Meer 2004; Maddocks and Oyston 2008). LTTRs were firstly described as transcriptional activators; however, several LTTRs have been shown to act as repressors (Jourlin-Castelli et al. 2000; Kim et al. 2003), and some LTTRs, such as GltC, act both as activators and repressors (Picossi et al. 2007).

LTTRs controlling the catabolism of aromatic compounds are co-inducer-responsive transcriptional regulators that have been described exclusively as activators (Table 1) and that were associated initially with the classic β-ketoadipate pathway. Some examples include CatR and ClcR involved in the degradation of catechol and chlorocatechol, respectively, in *P. putida* (McFall et al. 1998); BenM/CatM and SalR involved in benzoate and salicylate degradation, respectively, by the soil bacterium *Acinetobacter baylyi* strain ADP1 (Collier et al. 1998; Jones et al. 2000; Vaneechoutte et al. 2006); and PcaQ from *Agrobacterium tumefaciens* controlling the conversion of protocatechuate into β-ketoadipate (Parke 1996) (Table 1). However, it is now evident that LTTRs control a broad spectrum of aromatic degradation pathways (Table 1). For instance, HcaR regulates the expression of *hca* genes for the initial catabolism of 3-phenylpropionic acid in *E. coli* K12 (Díaz et al. 1998), TsaR regulates the *tsa* operon encoding the first steps in the degradation of *p*-toluenesulfonate in *Comamonas testosteroni* T-2 (Tralau et al. 2003b), AphT controls the *meta*-cleavage pathway of phenol degradation in *Comamonas testosteroni* TA441 (Arai et al. 2000), and DntR activates the expression of the *dnt* genes for 2,4-DNT degradation in *Burkholderia* sp. DNT (de las Heras et al. 2011). Recently, the DbdR protein from *Thauera aromatica* strain AR-1 was shown to control the

Table 1 Some examples of regulatory proteins of aromatic catabolic pathways in bacteria

Regulator[a]	Family	Microorganism	Activity[a]	Pathway[b]	Accession no.
CatM	LysR	*A. baylyi* ADP1	Activator	Catechol	P07774
ClcR	LysR	*R. opacus* 1CP (pAC25)	Activator	3-chlorobenzoate	AAC38250
CatR	LysR	*P. putida*	Activator	Catechol	A35118
CatR	LysR	*A. lwoffi*	Activator	Phenol/Catechol	O33945
CdoR	LysR	*Comamonas* sp. JS765	Activator	Catechol	AAC79916
AphT	LysR	*C. testosteroni* TA441	Activator	Phenol	BAA88500
NahR	LysR	*P. putida* (pNAH7)	Activator	Naphthalene/Salicylate	A31382
NahR	LysR	*P. stutzeri* AN10	Activator	Naphthalene/Salicylate	AAD02145
SalR	LysR	*A. baylyi* ADP1	Activator	Salicylate	AAF04311
PhnS	LysR	*Burkholderia* sp. RP007	Activator	Phenanthrene/Naphthalene	AAD09867
TcbR	LysR	*Pseudomonas* sp. P51 (pP51)	Activator	Trichlorobenzene	A38861
CbnR	LysR	*R. eutropha* NH9	Activator	Chlorocatechol	BAA74529
PcaQ	LysR	A. tumefaciens A348	Activator	Protocatechuate (*ortho*-cleavage)	AAA91130
LigR	LysR	*Sphingobium* sp. SYK-6	Activator	Protocatechuate (*meta*-cleavage)	BAB88739
BenM	LysR	*A. baylyi* ADP1	Activator	Benzoate	AAC46441
TfdT	LysR	*R. eutropha* JMP134	Activator	3-,4-chlorocatechol(2-,3-chlorobenzoate)	AAC44724
NtdR	LysR	*Acidovorax* sp. JS42	Activator	2,4- and 2,6-dinitrotoluene	AAP70492
DntR	LysR	*Burkholderia* sp. DNT	Activator	2,4-dinitrotoluene	AAP70493
NbzR	LysR	*Comamonas* sp. JS765	Activator	Salicylate, anthranilate	AAL76198
LinR	LysR	*Sphingomonas paucimobilis* UT26	Activator	2,5 and 2,6-dichlorohydroquinone	BAA36280
DbdR	LysR	*Thauera aromatica* AR-1	Activator	*3,5-dihydroxybenzoate* (*α– resorcylate; anaerobic*)	AIO06107
ThnR	LysR	*Sphingopysis granuli* TFA	Activator	Tetralin	AAU12855
Orf3	LysR	*Pseudomonas* sp. HR199	Activator	4-hydroxybenzoate	Y18527
HcaR	LysR	*E. coli* K-12	Activator	3-phenylpropionic acid	Q47141
TdnR	LysR	*P. putida* UCC22 (pTDN1)	Activator	Aniline	BAA12810
TsaR	LysR	*C. testosteroni* T-2	Activator	*p*-toluene sulfonate	AAC44806
PucR	LysR	*B. subtilis*	Activator	Purines	O32146
AtzR	LysR	*Pseudomonas* sp. ADP	Activator	Cyanuric acid	NP_862536

XylR	NtrC	*P. putida* mt-2 (pWW0)	Activator	Toluene	AAA26028
TouR	NtrC	*P. stutzeri* OX1	Activator	Toluene	CAB52211
TbuT	NtrC	*B. pickettii* PKO1	Activator	Toluene	AAC44567
TbmR	NtrC	*Burkholderia* sp. JS150	Activator	Toluene	
DmpR	NtrC	*Pseudomonas* sp. CF600 (pVI150)	Activator	Phenol	A47078
PhhR	NtrC	*P. putida* P35X	Activator	Phenol	S47095
AphR	NtrC	*C. testosteroni* TA 441	Activator	Phenol	BAA34177
MopR	NtrC	*A. calcoaceticus* NCIB8250	Activator	Phenol	CAA93242
MphR	NtrC	*A. pittii* PHEA-2	Activator	Phenol	ACO88925
PhlR	NtrC	*P. putida* H (pPGH1)	Activator	Phenol	CAA62584
PhlR	NtrC	*R. eutropha* JMP134	Activator	Phenol	AAC77386
PhcR	NtrC	*C. testosteroni* R5	Activator	Phenol	BAA87867
PoxR	NtrC	*R. eutropha* E2	Activator	Phenol	AAC32451
PheR	NtrC	*P. putida* BH	Activator	Phenol	D63814
PdeR	NtrC	*T. aromatica* K172	Activator	*Phenol (anaerobic)*	CAC12685
PdeR	NtrC	"*Aromatoleum aromaticum*" EbN1	Activator	*Phenol (anaerobic)*	CAI07889
PhnR	NtrC	*Burkholderia* sp. RP007	Activator	Phenanthrene/Naphthalene	AAD09866
TmbR	NtrC	*P. putida* TMB	Activator	Trimethylbenzene	U41301
HbpR	NtrC	*P. azelaica* HBP1	Activator	2-hydroxybiphenyl	AAA84988
BphR	NtrC	*S. aromaticivorans* F199 (pLN1)	Activator	Biphenyl	AAD03979
AreR	NtrC	*A. baylyi* ADP1	Activator	Aryl esters	AF1509028
EtpR	NtrC	"*Aromatoleum aromaticum*" EbN1	Activator	*p-ethylphenol/ p-hydroxyacetophenone (anaerobic)*	CAI06292
XylS	AraC/XylS	*P. putida* mt-2 (pWW0)	Activator	3-methylbenzoate/Benzoate	AAA26029
BenR	AraC/XylS	*P. putida*	Activator	Benzoate	AF218267
HpaA	AraC/XylS	*E. coli* W	Activator	3-, 4-hydroxyphenylacetate	Z37980
PobR	AraC/XylS	*Azotobacter chroococcum*	Activator	4-hydroxybenzoate	AAF03756
MaoB	AraC/XylS	*E. coli* K-12	Activator	Tyramine	BAA11058
IpbR	AraC/XylS	*P. putida* RE204	Activator	Isopropylbenzene	AF006691
CbdS	AraC/XylS	*Burkholderia* sp. TH2	Activator	2-halobenzoate	BAB21583

(*continued*)

Table 1 (continued)

Regulator[a]	Family	Microorganism	Activity[a]	Pathway[b]	Accession no.
OxoS	AraC/XylS	*P. putida* 86	Activator	Quinoline	CAA73202
AntR	AraC/XylS	*P. resinovorans* CA10	Activator	Carbazole, anthranilate	BAC41529
IifR	AraC/XylS	*A. baumannii*	Activator	Indole	ENW75084
PheR	AraC/XylS	*R. erythropolis*	Activator	Phenol	CAJ01323
AraC	AraC/XylS	*Mycobacterium gilvum* PYR-GCK	Activator	Pyrene	ABP47731
FeaR	AraC/XylS	*E. coli*	Activator	Phenylethylamine	CTY06606
AadR	CRP/FNR	*R. palustris*	Activator	*Benzoate/4-hydroxybenzoate/ Cyclohexanecarboxylate (anaerobic)*	Q01980
HbaR	CRP/FNR	*R. palustris*	Activator	*4-hydroxybenzoate (anaerobic)*	AAF04013
CprK	CRP/FNR	*D. hafniense*	Activator	*o-chlorophenol (anaerobic)*	AAL87770
GlxR	CRP/FNR	*Corynebacterium glutamicum*	Repressor	3-hydroxybenzoate/Gentisate	Q79VI7
CRP	CRP/FNR	*E. coli* K-12	Activator	3-hydroxyphenylpropionate/ 4-hydroxyphenylacetate/phenylacetate	J01598
AcpR	CRP/FNR	*Azoarcus* sp. CIB	Activator	*Benzoate/3-methylbenzoate (anaerobic)*	AAY81959
CymR	TetR	*P. putida* F1	Repressor	*p*-cymene	AAB62296
PaaR	TetR	*C. glutamicum*	Repressor	Phenylacetate	CCF55037
PaaR	TetR	*T. thermophilus* HB8	Repressor	Phenylacetate	BAD70796
PaaR	TetR	*Streptomyces pristinaespiralis*	Repressor	Phenylacetate	EDY64415
PfmR	TetR	*T. thermophilus* HB8	Repressor	Phenylacetate	PDB: 3VPR_D
HdnoR	TetR	*A. nicotinovorans*	Repressor	Nicotine	ABA41004
NicS	TetR	*P. putida*	Repressor	Nicotinate	Q88FX7
RolR	TetR	*C. glutamicum*	Repressor	Resorcinol	BAB97394
MbdR	TetR	*Azoarcus* sp. CIB	Repressor	*3-methylbenzoate (anaerobic)*	CCH23038
BzdR	BzdR	*Azoarcus* sp. CIB	Repressor	*Benzoate (anaerobic)*	AAQ08805
BoxR	BzdR	*Azoarcus* sp. CIB	Repressor	Benzoate	CCD33120
PaaX	GntR	*E. coli* W	Repressor	Phenylacetate	CAA66101
PaaN	GntR	*P. putida* U	Repressor	Phenylacetate	AF029714
BphS	GntR	*R. eutropha* A5	Repressor	Biphenyl/4-chlorobiphenyl	CAC05302
BphR1	GntR	*P. pseudoalcaligenes* KF707	Activator	Biphenyl	BAA12882
AphS	GntR	*C. testosteroni* TA441	Repressor	Phenol	BAA8295
PhcS	GntR	*C. testosteroni* R5	Repressor	Phenol	BAB61103

VanR	GntR	*A. baylyi* ADP1	Repressor	Vanillate	O24839
VanR	GntR	*P. putida* WCS358	Repressor	Vanillate	AJ252091
VanR	GntR	*Caulobacter crescentus* CB15	Repressor	Vanillate	AAK24363
$CarR_{13}$	GntR	*Janthinobacterium* sp. 13	Repressor	Carbazole	BAC56739
MeqR2	GntR	*Arthrobacter* sp. Rue61a	Repressor	Quinaldine	AFR31129
PbaR	IclR	*Sphingobium wenxiniae* JZ-1T	Activator	3-phenoxybenzoate	A0A0K2CTW1
IphR	IclR	*Comamonas* sp. E6	Represor	Isophthalate	C4TNS6
TphR	IclR	*Comamonas* sp. E6	Activator	Terephthalate	AB238679
MhpR	IclR	*C. testosteroni* TA441	Activator	3-hydroxyphenylpropionate	Q9S159
MhpR	IclR	*E. coli* K-12	Activator	3-hydroxyphenylpropionate	P77569
TsaQ	IclR	*C. testosteroni* T-2	Activator	*p*-toluenesulfonate	Q6XL52
PobR	IclR	*A. baylyi* ADP1	Activator	4-hydroxybenzoate	Q43992
PcaU	IclR	*A. baylyi* ADP1	Activator/Repressor	Protocatechuate	AAC37157
PcaR	IclR	*P. putida* PRS2000	Activator	Protocatechuate	Q52154
HmgR	IclR	*P. putida* KT2440	Repressor	Homogentisate	Q88E46
TsdR	IclR	*R. jostii* RHA1	Repressor	γ-Resorcylate	Q0SFL4
OphR	IclR	*Rhodococcus* sp DK17	Repressor	Phthalate	A4ZXZ2
CatR	IclR	*R. erythropolis* CCM2595	Repressor	Catechol	T1VW59
NpdR	IclR	*Rhodococcus opacus* HL PM-1	Repressor	2,4,6-trinitrophenol (picric acid)	Q9AH06
GenR	IclR	*C. glutamicum*	Activator	3-hydroxybenzoate /Gentisate	Q8NLB8
HpaR	MarR	*E. coli* W	Repressor	Homoprotocatechuate	AFH14229
CbaR	MarR	*C. testosteroni* BR60	Repressor	Chlorobenzoate	NP_869726
NbzR	MarR	*P. putida* HS12 (pNB1)	Repressor	Aminophenol	AAK26517
HcaR	MarR	*A. baylyi* ADP1	Repressor	*p*-hydroxycinnamates	AAP78949
CouR	MarR	*R. jostii* RHA1	Repressor	*p*-hydroxycinnamates	PDB: 5CYV_B
CouR	MarR	*R. palustris*	Repressor	*p-coumarate (anaerobic)*	CAE27235
FerR	MarR	*P. fluorescens* BF13	Repressor	Ferulate	CAD60265
FerC	MarR	*Sphingobium* sp. SYK-6	Repressor	Ferulate	SLG_25040
PcaV	MarR	*Streptomyces coelicolor*	Repressor	Protocatechuate (*ortho*-cleavage)	PDB: 4G9Y_A
GenR	MarR	*C. testosteroni* CNB-1	Repressor	Gentisate	ACY33523
IacR	MarR	*Acinetobacter baumannii*	Repressor	Indole-3-acetate	
BadR	MarR	*R. palustris*	Repressor	*Cyclohexanecarboxylate (anaerobic)*	CAE26099

(*continued*)

Table 1 (continued)

Regulator[a]	Family	Microorganism	Activity[a]	Pathway[b]	Accession no.
NicR	MarR	*P. putida* KT2440	Repressor	Nicotinate	Q88FY0
BadM	Rrf2	*R. palustris*	Repressor	*Benzoate (anaerobic)*	CAE26107
BgeR	Rrf2	*Geobacter bemidjiensis*	Repressor	*Benzoate (anaerobic)*	ACH38458
VanR	PadR	*C. glutamicum*	Repressor	Vanillate	NP_601583

[a] Activators are indicated in green. Repressors are indicated in red
[b] Anaerobic pathways are indicated in italics

expression of the genes involved in the 3,5-dihydroxybenzoate (alpha-resorcylate) anaerobic degradation pathway, thus expanding the scope of LTTRs to the control of the anaerobic metabolism of aromatic compounds (Molina-Fuentes et al. 2015).

LTTRs involved in the degradation of aromatic compounds typically activate divergently transcribed catabolic promoters in response to inducers, usually intermediates of the corresponding catabolic pathways, and they repress their own synthesis (Schell 1993; Tropel and van der Meer 2004; Maddocks and Oyston 2008). This preference for an intermediate of the catabolic pathway rather than by the initial substrate of the pathway has been suggested as a mechanism to avoid gratuity expression. An unprecedented example of a regulatory system to prevent gratuitous induction has been described for the tetralin biodegradation genes (*thn*) in *Sphingopyxis granuli* strain TFA. ThnR is an LTTR that activates transcription in response to tetralin, but its activity is under the control of ThnY (López-Sánchez et al. 2009; García et al. 2011). ThnY is an iron-sulfur flavoprotein which, in the absence of an efficient substrate acting as an electron sink, is reduced by the ThnA3 ferredoxin of the tetralin dioxygenase complex avoiding expression of the *thn* genes irrespective of the ThnR activation by gratuitous inducers (Ledesma-García et al. 2016). This scenario occurs in the presence of an inducer molecule that is not a substrate for the dioxygenase (deficient electron flux to the dioxygenase) or when the concentration of the inducer is very low (Ledesma-García et al. 2016).

Aromatic compound responsive LTTRs bind within the target promoter, independently of the presence of the inducer molecule, to a long sequence of approximately 50–60 bp which contains two distinct sites, RBS and ABS. RBS (recognition binding site) is a high-affinity binding site centered at position −66 and encompassing a characteristic inverted repeat motif including a T-N11-A consensus sequence. ABS (activation binding site) is a low-affinity binding site with half-dyad symmetry and located at positions −27 to −32. Type I LTTRs bind to ABS only in the presence of the inducer molecule in contrast to type II LTTRs which bind ABS irrespective of inducer. Based on this plasticity, the so-called sliding dimer model has been proposed as the mechanism of activation of the target promoter by LTTRs. This mechanism has been well studied in AtzR, an LTTR responsible for activation of the *atzDEF* cyanuric acid utilization operon in *Pseudomonas* sp. ADP (Porrúa et al. 2007). According to this model, in the absence of inducer, a type I LTTR dimer binds only the RBS site, while the ABS site is also occupied by a second dimer in type II LTTRs, causing in both cases a DNA bending. A conformational change upon inducer binding causes a shift from a more proximal ABS subside to a more distal ABS subside in type II LTTRs or the occupancy of the ABS site by the second dimer in type I LTTRs. In both cases, a relaxation of the DNA bending allows the formation of an active complex with the RNAP leading to transcription activation (Tropel and van der Meer 2004; Porrúa et al. 2007; Maddocks and Oyston 2008). However, an activation mechanism based on bend induction rather than bend relaxation has been found in LigR, an LTTR involved in regulation of the protocatechuate *meta*-cleavage pathway in *Sphingobium* sp. strain SYK-6 (Kamimura et al. 2010). Thus, the mechanistic versatility of LTTRs is still far to be completely understood.

LTTRs act as tetramers in its biologically active form; however, variable oligomeric states, ranging from monomers to homotetramers, are found when LTTRs are in solution (Schell 1993, Tropel and van der Meer 2004; Maddocks and Oyston 2008). LTTRs display two domains, an N-terminal domain that contains a winged helix-turn-helix (wHTH) motif for DNA binding and a C-terminal domain that provides the effector-binding and multimerization functions. The three-dimensional structures of the C-terminal domains of BenM and CatM have been determined and were found to consist of nine α-helices and nine β-strands with Rossmann-like folds (Ezezika et al. 2007). Other aromatic responsive LTTRs, such as DntR, appear to have similar structure (Smirnova et al. 2004). The first full-length LTTR crystal structure resolved was that of CbnR which controls the degradation of chlorocatechols in *Ralstonia eutropha* NH9 (Ogawa et al. 1999; Muraoka et al. 2003). CbnR was crystallized as a tetramer consisting of two dimers. Each dimer is composed by a short-form and an extended-form subunit (protomers), resulting in a tetrameric molecule with asymmetrical ellipsoidal shape. The increasing number of full-length LTTR structures resolved in the last years has contributed to a better understanding of the structure-function relationships of this group of transcriptional regulators (Monferrer et al. 2010). The protomers are composed of two domains, the N-terminal domain which harbors the DNA-binding elements and a C-terminal domain responsible of binding to inducer and connected by a large linker helix. Two different conformations, extended and compact, were found in the protomers of the asymmetric dimer (Monferrer et al. 2010). The full-length structure of DntR has revealed that while apo-DntR maintains an inactive compact configuration in solution, the inducer-bound holo-DntR adopts an expanded conformation. These observations are consistent with the known shifting of LTTR DNA-binding sites upon activation and the consequent relaxation in the bend of the promoter-operator region DNA, thus strongly supporting the sliding dimer model of activation proposed for LTTRs (Lerche et al. 2016).

4 Aromatic Compound Responsive Regulators of the NtrC Family

NtrC-like regulators are activators of promoters that utilize the alternative sigma factor σ^{54}. The σ^{54} promoters display a particular architecture defined by a highly conserved −12/−24 sequence recognized by the σ^{54}-RNAP holoenzyme, upstream activator sequences (UASs) situated more than 100 bp upstream the transcriptional start site and that bind to the NtrC-like regulator, and a DNA intrinsic or protein-induced curvature that promote the DNA looping required for the specific contact between the activator and the σ^{54}-RNAP subunit (Beck et al. 2007). Typically, NtrC-like activators consist of three different domains: (i) an N-terminal regulatory domain, (ii) a central domain responsible of multimerization into a hexamer and of ATP-hydrolyzing (activating) activity, and (iii) a C-terminal DNA-binding domain. Upon effector binding, NtrC regulators oligomerize and bind to the UAS. After the loop formation and the contact between the activator and σ^{54}-RNAP holoenzyme are

established, the energy produced by ATP hydrolysis is invested in remodeling the transcription complex from its closed configuration into an open transcriptionally active form (Bush and Dixon 2012).

Several NtrC-like activators respond to aromatic compounds (Table 1), but the best characterized are the XylR and DmpR proteins from *Pseudomonas* strains. XylR is the transcriptional regulator of the upper operon controlled by the *Pu* promoter and that encodes the upper route of the TOL catabolic pathway for toluene, *m*-xylene, and *p*-xylene degradation in *P. putida* (Ramos et al. 1997, Galvão and de Lorenzo 2006). The N-terminal domain of XylR generates an intramolecular repression on the central activating domain of the protein. Two additional domains described in XylR are a Q-linker of 20 residues that might be involved in protein oligomerization and a DNA-binding domain that shares similarity to that of the Fis protein, a well-known global regulator (Garmendia and de Lorenzo 2000; O'Neill 2001). The binding to the N-terminal domain of XylR of a surprising variety of alkylbenzene effectors (Galvão and de Lorenzo 2006) releases its repression on the activating domain and enables the binding to the target promoter and ATP hydrolysis (Perez-Martin and de Lorenzo 1995), which constitutes the molecular basis for the activation of the σ^{54}-dependent *Pu* promoter. The *xylR* gene is transcribed from the *Pr* promoter, and XylR levels in the cell are negatively regulated at the transcriptional level by a XylR self-repression that requires the participation of the IHF host factor forming an unusual feed-forward regulatory loop (Guantes et al. 2015). Moreover, XylR production is also subject of a complex posttranscriptional mechanism in which the so-called catabolic repressor control protein (Crc) acts as a translational co-repressor along with the RNA-binding factor Hfq (Moreno et al. 2015). Recently it was shown that the levels of the global regulators IHF and Crc are subject to growth phase-dependent control which in turn originates a bimodal regime of *Pu* expression in exponential phase, where a fraction of the population remains inactive at any one time after induction by *m*-xylene, and an unimodal response in stationary phase, where the whole population is induced at comparable rates. These results highlight the importance of cell physiology and internal composition and its impact on phenotypic variability that may be advantageous in competitive environmental settings (Guantes et al. 2015).

The ability of XylR to recognize several alkylbenzene compounds has been exploited to develop biosensors for BTEX, the more abundant aromatic mixture in the oil industry (Kim et al. 2005; de las Heras and de Lorenzo 2011). XylR was also engineered using synthetic biology approaches to detect nitrotoluenes for bio-detection of landmines (Garmendia et al. 2008; de las Heras and de Lorenzo 2011). Novel XylR variants that allow the implementation of single Boolean logic operation were also generated, and they can be used for biosensor development (Calles and Lorenzo 2013).

DmpR and PhlR are closely related NtrC-like regulators of aerobic phenol catabolism in *Pseudomonas* (Table 1). DmpR has been extensively studied, and it regulates the catabolism of phenols and methyl-phenols in *Pseudomonas* sp. strain CF600 by controlling the transcription of the σ^{54}-dependent *Po* promoter that drives the expression of the catabolic *dmp*-operon (Shingler 2004; Gupta et al. 2012). The nonoverlapping σ^{70}-dependent promoter (*Pr*) controls the production of DmpR.

Transcription-driven supercoiling arising from the σ^{54}-promoter allows inter-promoter communication that results in stimulation of the activity of the σ^{70}-promoter without it possessing a cognate binding site for the σ^{54}-RNAP holoenzyme. This mode of control has the potential to be a prevalent, but hitherto unappreciated, mechanism by which bacteria adjust promoter activity to gain appropriate transcriptional control (del Peso-Santos and Shingler 2016). Recently, it was shown that the 5′-leader region (5′-LR) of the *dmpR* gene functions as a regulatory hub to control DmpR levels by two distinct mechanisms. At the level of transcription, inhibition of full-length transcripts was traced to an A-rich DNA-binding motif located downstream of the *Pr* promoter. At the translational level, Hfq aids Crc to bind to a catabolite activity motif overlapping the ribosome-binding site at the mRNA facilitating the Crc-dependent repression in intact cells. Interestingly, the entire 5′-LR of *dmpR* is highly conserved in closely related phenolic catabolic systems, suggesting a strong evolutionary pressure to maintain these regulatory motifs as well as additional potential regulatory features that remain to be elucidated (Madhushani et al. 2014).

Although the molecular architecture of DmpR is similar to that of XylR, they show different effector specificities (Galvão and de Lorenzo 2006), and the residues involved in effector recognition are confined to a stretch of 75 amino acids defined as the effector-specifying region (Skarfstad et al. 2000). 3D models of XylR and DmpR predicted structural features for shaping an effector-binding pocket and interaction with the central domain (Suresh et al. 2010). Three other phenolic compound-sensing NtrC-like regulators are those controlling the anaerobic degradation of phenol in *Thauera aromatica* K172 and *Aromatoleum aromaticum* EbN1 (PdeR) and the one involved in the anaerobic *p*-ethylphenol degradation in *A. aromaticum* EbN1 (EtpR) (Table 1) (Breinig et al. 2000; Wöhlbrand et al. 2007; Büsing et al. 2015). All these five regulators share eight residues that may be involved in the recognition of the phenolic moiety of the effector molecule (Büsing et al. 2015).

Other examples of regulators of the NtrC family are TbuT and TbmR, which control toluene monooxygenase gene expression in two strains of *Burkholderia* (Byrne and Olsen 1996; Leahy et al. 1997); TouR, which controls the degradation of phenol and toluene in *Pseudomonas stutzeri* OX1 (Solera et al. 2004); and AreR, which is involved in the aryl ester degradation pathway in *Acinetobacter baylyi* ADP1 (Jones and Williams 2001) (Table 1). Cross talk regulation has been demonstrated between XylR/DmpR and TbuT/TbmR for activation of their mutual promoters while maintaining their inducer specificity (Leahy et al. 1997; Arenghi et al. 1999).

5 Aromatic Compound Responsive Regulators of the AraC/XylS Family

Members of the AraC/XylS family have two structural domains, i.e., the C-terminal DNA-binding domain and the N-terminal signaling domain, connected by a relatively unstructured linker (Seedorff and Schleif 2011). The more variable N-terminal region is responsible for cofactor binding and/or multimerization. The C-terminal

domain includes two tetra-helical HTH DNA-binding motifs. One or both HTH motifs bind DNA upstream, and sometimes downstream, of the target promoters (Seedorff and Schleif 2011).

The AraC/XylS family members responsible for the control of the catabolism of aromatic compounds are widely distributed in prokaryotes (Table 1). The XylS protein is the best characterized AraC member that controls the expression of an aromatic catabolic pathway. The pWW0-encoded XylS regulator mediates transcriptional activation of the *Pm* promoter driving the expression of the *meta*-pathway genes in *P. putida* mt-2, in response to 3-methylbenzoate (*m*-toluate) and benzoate as inducers (Gallegos et al. 1993). In addition to its known influence favoring protein dimerization, the effector is able to modify XylS conformation to trigger N-terminal domain intramolecular derepression (Domínguez-Cuevas et al. 2008). It has been suggested that the presence of the effector *m*-toluate triggers a cell response similar to the heat-shock response (Marqués et al. 1999), which explains that XylS-mediated transcription activation from the *Pm* promoter is driven by the σ^{32} heat-shock sigma factor in the early exponential growth phase. Activation of *Pm* transcription is achieved through a switch to the σ^{38} sigma factor when cultures reach the stationary phase (Marqués et al. 1999). By using a recombinant XylS-CTD soluble monomeric variant devoid of the N-terminal domain, it was shown that binding to *Pm* occurred sequentially. Firstly, a XylS-CTD monomer binds to the proximal site overlapping the RNAP-binding sequence to form complex I. This first event increased *Pm* bending to 50 degrees and was followed by the binding of the second monomer, which further increased the observed global curvature to 98 degrees (Domínguez-Cuevas et al. 2010). Despite the lack of information about the structure of XylS, mutagenesis studies have successfully generated regulators with altered inducer specificity (Michán et al. 1992).

BenR is a XylS-like activator able to trigger the activity of *Pben* promoter by recognition of benzoate as effector, allowing the expression of the *ben* operon that encodes the benzoate dioxygenase which converts benzoate into catechol in *P. putida* (Table 1) (Cowles et al. 2000). The N-terminal regions of BenR and XylS share about 65% amino acid identity, and both regulators respond to benzoate as an effector molecule (Cowles et al. 2000). The similarity also extends to their C-terminal DNA-binding domains suggesting that cross activation of their target promoters could take place. In fact, the ability of BenR to activate the *Pm* promoter of the *meta*-cleavage pathway operon of the TOL catabolic plasmid in response to benzoate has been described. Therefore, BenR behaves as an activator of benzoate degradation via *ortho*-ring fission, as an activator of benzoate and methylbenzoate degradation via *meta*-ring fission, and it is also involved in the benzoate-dependent repression of 4-hydroxybenzoate degradation by controlling the expression of the *pcaK* gene (encodes the 4-hydroxybenzoate transporter) in *P. putida* (Cowles et al. 2000). Although the cross activation of *Pben* by XylS had been previously shown (Cowles et al. 2000; Domínguez-Cuevas et al. 2006), these studies were performed using multicopy *Pben-lacZ* transcriptional fusions. In fact, any cross activation of *Pben* promoter by XylS will cause a metabolic conflict during the degradation of *m*-xylene because the produced 3-methylbenzoate could be channeled through the

ortho-pathway and generate toxic dead-end metabolites. Recently, it was shown that the natural expression ranges of XylS are insufficient to cause a significant cross regulation of *Pben* if cells face either endogenous or exogenous 3-methylbenzoate. This lack of cross regulation relies on the fact that the *Pben* promoter has evolved to avoid a strong interaction with XylS, likely by lacking the A box in the proximal operator. This scenario reveals how a simple genetic tinkering facilitates the recruitment of catabolic pathways (the *meta*-pathway) in a host that harbors a non-fully compatible metabolism (the *ortho*-pathway) and suggests strategies for orthogonalization of new pathways implanted in a pre-existing metabolic chassis (Pérez-Pantoja et al. 2015).

Some other examples of AraC/XylS family members involved in the control of aromatic catabolic pathways (Table 1) are: PobR controls the *p*-hydroxybenzoate hydroxylase in many bacteria (Quinn et al. 2001; Donoso et al. 2011); OxoS is required for quinoline-dependent growth of *P. putida* 86 (Carl and Fetzner 2005); AntR controls the expression of the *antABC* operon coding for anthranilate 1,2-dioxygenase as well as of the *car* operon involved in the conversion of carbazole to anthranilate in *P. resinovorans* strain CA10 (Urata et al. 2004); HpaA regulates the *hpaBC* operon of *E. coli* W, which produces the hydroxylase activity for the catabolism of 4-hydroxyphenylacetic acid, in response to this aromatic acid, 3-hydroxyphenylacetic acid, or phenylacetic acid (Prieto and García 1994); IifR activates de *iif* operon involved in the indole degradation in response to indole (Lin et al. 2015); PheR activates the *pheA2* promoter that controls phenol degradation genes in *Rhodococcus* strains (Szőköl et al. 2014).

6 Aromatic Compound Responsive Regulators of the CRP/FNR Family

CRP/FNR proteins stand out in responding to a broad spectrum of intracellular and exogenous signals such as cAMP, anoxia, redox state, oxidative and nitrosative stress, nitric oxide (NO), carbon monoxide (CO), 2-oxoglutarate, or temperature (Körner et al. 2003). Within the CRP/FNR superfamily, functionally distinct transcriptional regulators (both activators and repressors) have evolved based on a common modular design. The N-terminal domain comprises a β-barrel, responsible for ligand recognition, while the C-terminal domain possesses a four-stranded wHTH motif for DNA binding. Both parts are connected by an α-helix frequently implicated in protein dimerization (Townsend et al. 2014). To accomplish their roles, CRP/FNR members might also have prosthetic groups such as an iron-sulfur group or heme, designed for the interaction with oxygen, NO, or CO (Körner et al. 2003). Regardless of the common structure, however, allosteric networks leading to the regulator activation after ligand binding are diverse within the CRP-FNR superfamily. There are reported cases of both negative (Townsend et al. 2014) and positive (Levy et al. 2008) cooperativity for ligand binding, and also ligand-independent regulators have been described (Agari et al. 2012). In the last case, regulator abundance would determine the extent of the transcription regulation effect (Agari

et al. 2010). The vast majority of FNR-regulated promoters contain a consensus FNR-binding site centered around 41.5 bp upstream of the transcriptional start site, and they are termed class II FNR-dependent promoters (Busby and Ebright 1999).

Among the functions of CRP/FNR proteins is that of regulating the expression of metabolic pathways for the use of aromatic compounds (Table 1). Two of these proteins, HbaR and AadR, have been described in the anaerobic catabolism of aromatic compounds in *Rhodopseudomonas palustris*. HbaR regulates the anaerobic 4-hydroxybenzoate catabolism by activating the expression of the gene encoding the first enzyme of the pathway (4-hydroxybenzoate-CoA ligase) in the presence of the 4-HBA inducer (Egland and Harwood 2000). The expression of the *hbaR* gene is, in turn, under oxygen control since it requires activation by AadR under anaerobic conditions. Accordingly, AadR contains some of the essential conserved Cys residues for iron-sulfur coordination as in the FNR protein (Dispensa et al. 1992). AadR, together with the BadR protein (see below), also modulates the expression of genes involved in the anaerobic metabolism of benzoate and cyclohexanecarboxylate, thus representing an oxygen sensor that regulates anaerobic catabolism of aromatic compounds in *R. palustris* (Dispensa et al. 1992; Egland and Harwood 2000). At the top of this regulatory cascade is another CRP/FNR family member, the FixJ regulator, that controls the expression of the *aadR* gene (Rey and Harwood 2010).

In the β-proteobacterium *Azoarcus* sp. CIB, the AcpR protein is required for the expression of the *bzd* and *mbd* genes that encode the central pathways for the anaerobic catabolism of benzoate and 3-methylbenzoate, respectively (Table 1) (Durante-Rodríguez et al. 2006; Juárez et al. 2012). AcpR favors the activation of the P_N promoter, which drives the expression of the *bzd* genes, in the absence of oxygen through contacts with the σ^{70} and the α-subunit of the RNAP (Durante-Rodríguez et al. 2006). Despite the predicted structural similarity between FNR and AcpR, the two proteins do not have the same regulatory functions within the cell. Thus, whereas in *E. coli* the lack of the FNR protein has a pleiotropic effect on the expression of a moderate number of genes, the lack of AcpR in *Azoarcus* sp. CIB does alter the ability to catabolize aromatic compounds through the benzoyl-CoA pathway but does not affect the anaerobic growth on nonaromatic carbon sources. In this sense, the physiological role of AcpR in *Azoarcus* would be equivalent to that of AadR in *R. palustris* (Durante-Rodríguez et al. 2006).

In *E. coli* and *Rhodococcus* sp. TFB, CRP regulators have been reported to mediate carbon catabolite repression of several aromatic acids and tetralin catabolic pathways, respectively (Table 1) (Díaz et al. 2001; Torres et al. 2003; Tomás-Gallardo et al. 2012). In contrast to CRP from *E. coli* that acts as an activator in the absence of the preferred carbon source (glucose), CRP from *Rhodococcus* sp. TFB acts as a repressor in the presence of the preferred carbon source. A third CRP-type protein, GlxR, is also a global regulator that represents a central control point in the *Corynebacterium glutamicum* response to different nutrient sources. GlxR represses, among others, the genes for 3-hydroxybenzoate and gentisate metabolism, and it responds to cAMP levels (Table 1) (Chao and Zhou 2014; Townsend et al. 2014).

A branch of the CRP-FNR family, CprK proteins, includes transcriptional regulators that mediate the response to halogenated aromatic compounds in dehalorespiration of different strains of *Desulfitobacterium*. CprK proteins activate transcription from promoters containing a 14-bp inverted repeat (dehalobox) that closely resembles the FNR-box (Pop et al. 2006). The unusually high occurrence of CprK paralogs is likely to be correlated with the relatively large number of halogenated compounds that these organisms can accept as terminal electron acceptors, enabling a specific response by each regulator to a specific group of halogenated compounds (Gabor et al. 2008). In *Desulfitobacterium hafniense*, for instance, CprK1 induces expression of halorespiratory genes upon binding of *o*-chlorophenol ligands and is reversibly inactivated by oxygen through disulfide bond formation (Pop et al. 2006). Crystal structures of CprK1 in the ligand-free (both oxidation states), ligand-bound (reduced), and DNA-bound states allowed a complete structural description of both redox-dependent and allosteric molecular rearrangements (Levy et al. 2008).

7 Aromatic Compound Responsive Regulators of the IclR Family

The IclR family is an extended type of prokaryotic transcription regulators (activators, repressors, and proteins with a dual role) that have been described to be involved in the control of different bacterial processes (Krell et al. 2006; Molina-Henares et al. 2006; Chao and Zhou 2013). The N-terminal domain of these regulators comprises a wHTH DNA-binding motif responsible for its positioning on target promoters as a dimer or as a pair of dimers. However, no clear consensus exists on the architecture of DNA-binding sites within the IclR-targeted promoters (Cheng et al. 2015). The C-terminal domain of IclR-like regulators is the effector-binding domain and regulates subunit multimerization after recognition of the effector molecule. In case of working as transcriptional activators, IclR proteins bind to their target promoters in the absence of the effector molecule (DiMarco and Ornston 1994; Gerischer et al. 1998; Guo and Houghton 1999; Torres et al. 2003), but they need the inducer to recruit RNAP to the promoter (Guo and Houghton 1999). For IclR-negative regulators, transcription is prevented either by occluding the RNAP-binding site or by destabilizing the open complex, and the presence of the effector molecule abolishes that behavior (Yamamoto and Ishihama 2002).

An important number of IclR members regulate catabolic pathways for the degradation of aromatic compounds (Table 1). In *A. baylyi* ADP1 the PobR and PcaU proteins have been described to be indispensable for the induction of 4-hydroxybenzoate and protocatechuate metabolic pathways, respectively, and both of them act as repressors of their own expression (DiMarco and Ornston 1994; Gerischer et al. 1998; Trautwein and Gerischer 2001). Interestingly, it is known that PcaU acts on the promoter of the catabolic genes both as a transcriptional activator, in the presence of the cognate inducer (protocatechuate), and as a repressor, in the absence of the inducer (Popp et al. 2002). A homologue of PcaU, PcaR,

exists in *Pseudomonas putida*, working in this case exclusively as activator of the *pca* genes for protocatechuate degradation (Guo and Houghton 1999).

The MhpR protein is necessary for the induction of the genes responsible of 3-hydroxyphenylpropionic acid metabolism in *E. coli*. In contrast to most aromatic compound responsive IclR-type regulators, the expression of *mhpR* from *Pr* promoter is constitutive and independent of self-regulation. Moreover, MhpR seems to be essential for recruiting a second activator, the global cAMP receptor protein (CRP) regulator, to the cognate *Pa* catabolic promoter (Torres et al. 2003), a feature that has not been reported for other IclR-type regulators. A MhpR-like protein involved in 3-hydroxyphenylpropionic acid metabolism has also been described in *Comamonas testosteroni* (Arai et al. 1999). Two other IclR-type activators, i.e., TphR and TsaQ for terephthalate and *p*-toluenesulfonate metabolism, respectively, have been reported in *C. testosteroni* strains (Tralau et al. 2003a; Kasai et al. 2010). The PbaR activator from *Sphingobium wenxiniae* JZ-1 T controls the degradation of 3-phenoxybenzoate and is the only IclR-type regulator so far described that binds downstream to the translation start site of the regulated gene (Cheng et al. 2015).

In Gram-negative bacteria, most IclR-type regulators that control aromatic catabolic pathways behave as transcriptional activators. Some exceptions are the HmgR protein that controls homogentisate degradation in *P. putida* (Arias-Barrau et al. 2004) and the IphR regulator for isophthalate metabolism in *C. testosteroni* (Table 1) (Fukuhara et al. 2009). In contrast, in *Actinobacteria* most IclR-type regulators described behave as transcriptional repressors (Table 1), e.g., TsdR (γ-resorcylate pathway), OphR (phthalate pathway), CatR (catechol pathway), and NpdR (2,4,6-trinitrophenol pathway) in *Rhodococcus* strains (Nga et al. 2004; Veselý et al. 2007; Choi et al. 2015; Kasai et al. 2015); and only one transcriptional activator, the GenR regulator that controls the catabolism of 3-hydroxybenzoate and gentisate, was reported in *Corynebacterium glutamicum* (Chao and Zhou 2013).

8 Aromatic Compound Responsive Regulators of the TetR Family

The TetR family is well characterized and widely distributed in bacteria (Ramos et al. 2005). The 3D structure of the prototype TetR reveals the existence of two domains, a N-terminal domain which contains the tetra-helical HTH-DNA-binding motif and a C-terminal domain involved in effector (tetracycline) binding (Orth et al. 2000). Members of the TetR family exhibit a high conservation of sequences for the DNA-binding domain. The TetR family regulators are mostly repressors that bind their operators, composed of 10–30-bp palindromic sequences, to repress the target genes and are released from the DNA when bound to their cognate ligands (Ramos et al. 2005).

Some TetR-type regulators involved in the regulation of aromatic catabolic pathways have been described (Table 1). CymR is a transcriptional repressor involved in the control of the gene expression for *p*-cymene (*cym*) and *p*-cumate (*cmt*) degradation in *P. putida* F1 (Eaton 1997). The CymR protein is a dimer in

solution, and it imposes its repressing effect by inhibiting RNAP access to the promoter, being *p*-cumate the effector molecule that avoids binding of CymR to its operator site (Eaton 1997). The PaaR protein is a transcriptional repressor of the *paa* genes for phenylacetate degradation in *Thermus thermophilus* HB8. Phenylacetyl-CoA is the effector molecule for effective transcriptional derepression (Sakamoto et al. 2011). Moreover, it has been described a new regulator, PfmR, that weakly cross regulated PaaR in vitro and that has an additional function in regulating the fatty acid metabolism in strain HB8 (Agari et al. 2012). The X-ray crystal structure of the N-terminal DNA-binding domain of PfmR and the nucleotide sequence of the predicted PfmR-binding site are quite similar to those of the TetR family repressor QacR from *Staphylococcus aureus*. Similar to QacR, two PfmR dimers bound per target DNA. The center of the PfmR molecule contains a tunnellike pocket, which may be the ligand-binding site of this regulator (Agari et al. 2012). PaaR is also the repressor of the *paa* genes involved in phenylacetate catabolism, in *Corynebacterium glutamicum* (Chen et al. 2012). An imperfect palindromic motif (5-″-ACTNACCGNNCGNNCGGTNAGT-3″, 22 bp) was identified in the upstream regions of *paa* genes. In addition, GlxR-binding sites were found, and binding to GlxR was confirmed. Therefore, phenylacetate catabolism in *C. glutamicum* is regulated by the pathway-specific repressor PaaR, which responds to phenylacetyl-CoA, and also likely by the global transcription regulator GlxR. By comparative genomic analysis, orthologous PaaR regulons were identified in 57 species, including species of *Actinobacteria*, *Proteobacteria*, and *Flavobacteria* that carry phenylacetate utilization genes and operate by conserved binding motifs, suggesting that PaaR-like regulation might commonly exist in these bacteria (Chen et al. 2012). In this sense, PaaR-like proteins controlling the expression of *paa* genes for the catabolism of phenylacetate have been also described in *Burkholderia cenocepacia* (Yudistira et al. 2011) and proposed in *Azoarcus* strains (Mohamed et al. 2002). In *Streptomyces pristinaespiralis*, PaaR is also involved in controlling the expression of the *paa* genes and plays a positive role in the regulation of the biosynthesis of pristinamycin I by affecting the levels of phenylacetyl-CoA as a supply of L-phenylglycine, one of the seven amino acid precursors of this antibiotic (Zhao et al. 2015).

HdnoR is a transcriptional repressor of the 6-hydroxy-D-nicotine oxidase, and it is encoded on the catabolic plasmid pAO1 responsible for nicotine degradation in *Arthrobacter nicotinovorans* (Sandu et al. 2003). The inducers 6-hydroxy-D-nicotine and 6-hydroxy-L-nicotine prevent the binding of HdnoR to its operator site allowing the expression of the 6-hydroxy-D-nicotine oxidase (Sandu et al. 2003). RolR may represent the first member of a new subfamily of TetR proteins involved in resorcinol degradation in *Corynebacterium glutamicum* (Li et al. 2011), and it shows generally low sequence similarities to other TetR family members, especially at its C-terminal end (Li et al. 2011). A 29-bp operator *rolO* was located at the intergenic region of *rolR* and the catabolic *rolHMD* genes, and it contains two overlapping inverted repeats that are essential for RolR binding and repression of both operons. The binding of RolR to *rolO* was avoided by resorcinol and hydroxyquinol, which are the starting compounds of the resorcinol catabolic

pathway, leading to the induction of *rol* genes (Li et al. 2012). A novel resorcinol-inducible expression system based on the RolR regulator and the cognate promoter fused with the operator (*rolO*) has been developed for *Streptomyces* and other *Actinobacteria* (Horbal et al. 2014). NicS is a repressor that controls the expression of the *nicAB* genes responsible for the conversion of nicotinic acid to 6-hydroxynicotinic acid in *P. putida* (Jiménez et al. 2011). Both aromatic heterocycles behave as NicS inducers. Interestingly, the expression of *nicS* is under control of a second regulator, NicR, that responds to 6-hydroxynicotinic acid, thus generating a peculiar regulatory loop (see below) (Jiménez et al. 2011).

The MbdR protein is a transcriptional repressor of the *mbd* genes for 3-methylbenzoate degradation in *Azoarcus* sp. CIB (Juárez et al. 2015). The 3D structure of MbdR revealed a conformation similar to that of other TetR family transcriptional regulators. 3-Methylbenzoyl-CoA, the first intermediate of the catabolic pathway, but not benzoyl-CoA, was shown to interact with MbdR and avoid binding to the operator region at the target promoters, leading to derepression of *mbd* genes. These results highlight the importance of recruiting the MbdR-based regulatory circuit to evolve a distinct central catabolic pathway that is only induced for the anaerobic degradation of aromatic compounds that generate 3-methylbenzoyl-CoA as central intermediate (Juárez et al. 2015).

9 Aromatic Compound Responsive Regulators of the BzdR Subfamily

The BzdR-like proteins (Table 1) constitute a new subfamily of aromatic transcriptional regulators belonging to the widely distributed HTH-XRE family of transcriptional regulators that includes the well-known Cro and cI lambda repressors. BzdR is the transcriptional repressor of the *bzd* cluster responsible for the anaerobic catabolism of benzoate in *Azoarcus* strains (Barragán et al. 2005). The BzdR protein exhibits two domains separated by a linker region, i.e., the N-terminal domain with a tetra-helical HTH-DNA-binding motif similar to that of the lambda repressor and the C-terminal domain similar to shikimate kinases (Barragán et al. 2005). Benzoyl-CoA, the first intermediate of the anaerobic benzoate degradation pathway, is the effector molecule of BzdR. Benzoyl-CoA interacts with the C-terminal domain of BzdR and prevents the binding of this protein to the three operator regions of the target P_N promoter without affecting its oligomeric state. The linker region of BzdR is required to transfer the conformational changes induced by benzoyl-CoA to the DNA-binding domain. The predicted structures of the respective N- and C-terminal domains could be fitted into a 3D reconstruction of the BzdR homodimer obtained by electron microscopy (Durante-Rodríguez et al. 2010). BzdR has been proposed as a model to study the evolution of transcriptional regulators. In this sense, an active BzdR-like regulator was engineered by fusing the DNA-binding domain of BzdR to the shikimate kinase I of *E. coli*, supporting the notion that an ancestral shikimate kinase domain could have been involved in the evolutionary origin of BzdR (Durante-Rodríguez et al. 2013). On the other hand, the C-terminal domain of

BzdR has been fused to the N-terminal domain of CI protein of the lambda phage to design a chimeric regulator, termed Qλ, able to reprogram the lytic/lysogenic lambda phage decision according to the intracellular production of benzoyl-CoA in *E. coli* (Durante-Rodríguez et al. 2016).

BoxR is a transcriptional repressor of the *box* genes involved in the aerobic hybrid pathway to degrade benzoate via coenzyme A derivatives in bacteria (Valderrama et al. 2012). The BoxR protein shows a significant sequence identity to BzdR. In *Azoarcus* sp. CIB, the paralogous BoxR and BzdR regulators act synergistically to assure a tight repression of the *bzd* and *box* genes in the absence of the common intermediate and inducer molecule benzoyl-CoA. Moreover, the observed expression of the *box* genes under anaerobic conditions (Valderrama et al. 2012) may constitute an alternative oxygen-scavenging mechanism when the cells face low-oxygen tensions that could inactivate the highly oxygen-sensitive anaerobic reductase and also a strategy to rapidly shift to the aerobic degradation if oxygen levels become high.

10 Aromatic Compound Responsive Regulators of the GntR Family

The proteins of the GntR superfamily are 239–254 amino acids long and share a similar N-terminal wHTH DNA-binding domain. This output domain is coupled to the C-terminal effector-binding and oligomerization domain that responds to a range of stimuli in the form of different small molecules. The C-terminal domain imposes steric constraints on the DNA-binding domain, hence influencing the HTH motif and thus playing an important role in regulation (Hoskisson and Rigali 2009, Suvorova et al. 2015). The structural data show that FadR from *E. coli* and AraR from *B. subtilis* bind as dimers to the target DNA through their N-terminal domains, but only few base pairs are specifically recognized within the complex (van Aalten 2001, Xu et al. 2001, Jain and Nair 2012).

There are some GntR family members related with aromatic catabolic pathways (Table 1). Most of them behave as repressors in the absence of effector with the exception of BphR1 (Orf0) of *P. pseudoalcaligenes* KF707 which acts as a repressor of salicylate catabolic genes but activates its own expression and that of biphenyl catabolic genes (Fujihara et al. 2006). The repressors PhcS and AphS regulate the expression of the phenol degradation genes in *Comamonas testosteroni* strains R5 and TA441, respectively (Arai et al. 1999; Teramoto et al. 2001). The VanR protein represses the vanillate demethylase (*vanAB*) genes in *A. baylyi* ADP1 and in diverse *Pseudomonas* strains (Morawski et al. 2000). Expression of the *vanAB* operon is repressed by VanR and induced by vanillate as well as, to a smaller degree, by its reduced derivatives vanillin and vanillyl alcohol in *Caulobacter crescentus* (Thanbichler et al. 2007). BphS controls the biphenyl degradation in *Ralstonia eutropha* A5 (Mouz et al. 1999) and *Pseudomonas* sp. strain KKS102 (Ohtsubo et al. 2001). $CarR_{J3}$ protein binds to two operator sequences (TtGTAGAACAA) in the absence of its inducer, which was identified as 2-hydroxy-6-oxo-6-

(2″-aminophenyl)hexa-2,4-dienoate, an intermediate of the carbazole degradation pathway, and represses the *car* operon in *Janthinobacterium* sp. 13 (Miyakoshi et al. 2006).

PaaX-like regulators are transcriptional repressors that control phenylacetic acid degradation gene clusters in several *Proteobacteria* (García et al. 2000; del Peso-Santos et al. 2006). Since they are bigger and do not show a significant sequence similarity with other members of the GntR family, they may constitute a new GntR subfamily. The PaaX repressor from *E. coli* recognizes the operator palindromic sequence (TGATTC-$N_{26–28}$-GAATCa) (Ferrández et al. 2000; Galán et al. 2004; Kim et al. 2004). Phenylacetyl-CoA specifically inhibited binding of PaaX to the target sequences, confirming the first intermediate of the pathway as the true inducer (Ferrández et al. 2000). Whereas the mechanism of repression of PaaX on the regulatory *Px* and the catabolic *Pz* promoters involves competition with the RNAP binding, the catabolic *Pa* promoter appears to be controlled by PaaX at a later stage of the transcription initiation process (Fernández et al. 2013). The PaaX repressor links the catabolism of aromatic compounds with the metabolism of penicillins since it is also a repressor of the *pac* gene encoding the penicillin G acylase (Galán et al. 2004). A role for the PaaX regulator in repressing the expression of the *sty* genes for the catabolism of styrene in *Pseudomonas* sp. Y2 has been reported, suggesting that PaaX is a major regulatory protein in the phenylacetyl-CoA catabolon through its response to the levels of this central metabolite (del Peso-Santos et al. 2006). Crystallization and preliminary X-ray diffraction studies on some PaaX-like regulators have been reported (Rojas-Altuve et al. 2011). In *E. coli*, the *paaX* gene is co-transcribed with *paaY*, which encodes a thioesterase, forming a regulatory operon. The PaaY protein is necessary for the efficient degradation of phenylacetate in *E. coli*, and two different roles for this protein can be envisioned. At the metabolic level, PaaY helps to prevent that phenylacetic acid catabolism might collapse cell growth by hydrolyzing some CoA derivatives whose accumulation may lead to the inhibition of the first steps of the *paa* pathway (Teufel et al. 2012). Moreover, PaaY plays a second role by facilitating the induction of the *paa* genes likely by its thioesterase activity that reduces the amount of some CoA-derived intermediate (s) originated during the catabolism of PA and that may behave as antagonists of the effect caused by the phenylacetyl-CoA inducer molecule on the PaaX repressor. This regulatory function mediated by PaaY constitutes an additional regulatory checkpoint that makes the circuit that controls the transcription of the *paa* genes more complex than previously anticipated, and it could represent a general strategy present in most bacterial *paa* gene clusters that also harbor the *paaY* gene (Fernández et al. 2013).

MeqR2 is a PaaX-type transcriptional repressor involved in the regulation of the genes responsible of quinaldine catabolism in *Arthrobacter* sp. strain Rue61a. MeqR2 forms a dimer in solution and binds to a palindromic operator whose core sequence (TGACGNNCGTcA) does not resemble that of PaaX operators. As some other GntR family regulators, such as PaaX and FadR that bind CoA thioesters, MeqR2 shows a high specificity for anthraniloyl-CoA, a downstream metabolite of the Meq pathway for quinaldine degradation, as effector. A binding stoichiometry of

one effector molecule per MeqR2 monomer and a high affinity (K_D of 22 nM) were determined (Niewerth et al. 2012).

11 Aromatic Compound Responsive Regulators of the MarR Family

MarR-type regulators are relatively small proteins (148–196 amino acids), and their 3D structures reveal a common triangular shape with a wHTH-DNA-binding motif. These transcription factors are typically homodimers and bind to palindromic DNA operators located within the target promoters. In most cases, in the absence of ligand, apo-MarR proteins bind to specific DNA operators, and upon ligand binding they show diminished DNA affinity. Usually, MarR regulators are promiscuous and can accommodate a variety of aromatic ligands with not very high affinity (Grove 2013; Kim et al. 2016).

Some members of this family are involved in specific responses to aromatic compounds (Table 1). HpaR is the transcriptional repressor of the 3,4-dihydroxyphenylacetate (homoprotocatechuate) *hpa* cluster of *E. coli* W. The *hpaR* gene is located upstream and divergently oriented with respect to the catabolic operon. HpaR negatively regulates not only the expression of the *hpa-meta* operon but also its own expression, with homoprotocatechuate, 4-hydroxyphenylacetate, and 3-hydroxyphenylacetate being the inducer molecules (Galán et al. 2003). Two DNA operators, OPR1 and OPR2, have been identified in the intergenic region located between the *hpa-meta* operon and the *hpaR* gene. The binding of HpaR to OPR2 displays a clear cooperativity with OPR1 binding (Galán et al. 2003). The CbaR repressor controls the *cbaABC* operon of plasmid pBRC60 required for chlorobenzoate degradation in *C. testosteroni* BR60. 3-Chlorobenzoate and protocatechuate are effectors for CbaR, with their binding leading to derepression (Providenti and Wyndham 2001). NbzR is a repressor that regulates the *nbz* operon for aminophenol degradation encoded on plasmid pNB1 of *P. putida* HS12, but the chemical inducer for the pathway has not yet been identified (Park and Kim 2001).

HcaR is the repressor of the *hca* genes responsible for hydroxycinnamates degradation in *A. baylyi* ADP1, with hydroxycinnamoyl-CoA thioesters being the effector molecules (Parke and Ornston 2003). The crystal structure of the apo-HcaR protein was recently determined in complexes with hydroxycinnamates and a specific 23-bp palindromic DNA operator. HcaR appears to be a tetramer, a dimer of dimers, in solution, and each dimer binds separate DNA-binding sites (*hca1* and *hca2*) using probably a DNA-loop formation mechanism that interferes with RNAP binding. HcaR recognizes four different ligands, i.e., ferulate, *p*-coumarate, vanillin, and 3,4-dihydroxybenzoate (which are substrate, intermediates, and products of ferulic acid processing by *hca* gene products) using the same binding site and rendering this repressor unproductive in recognizing a specific DNA target. These studies are consistent with a mechanism of HcaR derepression based on stabilization of a compact protein conformation that is unproductive in recognizing and binding a specific DNA operator (Kim et al. 2016).

CouR (FerC, FerR) regulates *p*-hydroxycinnamates (e.g., ferulate, *p*-coumarate) catabolism in different bacteria such as *Sphingobium* sp. SYK-6 (Kasai et al. 2012), *R. palustris* (Hirakawa et al. 2012), *P. fluorescens* (Calisti et al. 2008), and *R. jostii* RHA1 (Otani et al. 2015). In these repressors, DNA binding is abolished by *p*-hydroxycinnamoyl-CoA, the first metabolite of the pathway, allowing expression of the catabolic *cou* genes. Recent structural data with the CouR protein from *R. jostii* RHA1 establish that the CouR dimer binds two *p*-coumaroyl-CoA molecules in nonequivalent configuration, but this ligand binding did not lead to a significant conformational change in the repressor. Interestingly, the anionic bulky CoA moiety of *p*-hydroxycinnamoyl-CoA prevents the binding of DNA by steric occlusion of key DNA-binding residues and charge repulsion of the DNA backbone (Otani et al. 2015). In *R. palustris*, *p*-coumarate is not only a carbon source but the precursor of an unusual acyl-homoserine lactone (HSL) quorum-sensing signal, *p*-coumaroyl-HSL. A quantitative proteome and microarray study suggested that at least 40 genes and their encoded proteins are upregulated during growth on *p*-coumarate compared to succinate. Some of these genes are regulated by *p*-coumaroyl-HSL and the transcription protein RpaR, and others are regulated by CouR. In this bacterium, CouR controls not only the expression of *couAB* genes for *p*-coumarate degradation but also transport systems that are likely involved in the uptake of *p*-coumarate and structurally related compounds into cells (Phattarasukol et al. 2012).

PcaV is a MarR family regulator that represses transcription of genes encoding the central β-ketoadipate pathway in *Streptomyces coelicolor*. Structural data revealed that PcaV binds the β-ketoadipate pathway substrate protocatechuate with a high affinity and in a 1:1 stoichiometry, leading to a change in protein conformation incompatible with DNA binding. PcaV exhibits an unusually high degree of ligand selectivity and is one of the few MarR homologues incapable of binding salicylate. The Arg15 residue is critical for coordinating the protocatechuate ligand and plays a key role in binding DNA, thus functioning as a gatekeeper residue for regulating PcaV transcriptional activity (Davis et al. 2013).

GenR is a MarR-type transcriptional regulator that, in the absence of effectors, represses the *gen* cluster encoding the gentisate pathway in *Comamonas testosteroni* CNB-1. When effectors such as gentisate, 3-hydroxybenzoate, and benzoyl-CoA are present, the GenR protein is released from its DNA-binding site, and the repression of transcription is abolished. The finding that benzoyl-CoA can be recognized as GenR effector explains why the gentisate dioxygenase was induced when CNB-1 grew on benzoate using the *box* aerobic hybrid pathway that activates benzoate to benzoyl-CoA (Chen et al. 2014). IacR regulates negatively the *iac* genes responsible for indole-3-acetate catabolism in *Acinetobacter baumannii*, being this aromatic acid the potential effector that induces *iac* expression (Shu et al. 2015). BadR has been recently reassigned as a repressor that controls the genes involved in cyclohexanecarboxylate degradation in *R. palustris*. Some of these genes are also involved in the anaerobic degradation of benzoate. 2-Ketocyclohexane-1-carboxyl-CoA, an intermediate of cyclohexanecarboxylate degradation, interacts with BadR to abrogate repression (Hirakawa et al. 2015).

In *P. putida* the NicR repressor controls three nicotinic acid inducible catabolic operons, i.e., *nicAB*, encoding the upper pathway that converts nicotinic acid into 6-hydroxynicotinic acid, *nicCDEFTP*, and *nicXR* operons, responsible for channeling the latter to the central metabolism, which are driven by the *Pa*, *Pc*, and *Px* promoters, respectively (Jiménez et al. 2011). The *nicR* regulatory gene encodes a MarR-like protein that represses the activity of the divergent *Pc* and *Px* promoters being 6-hydroxynicotinic acid the inducer molecule. An additional gene, *nicS*, which is associated to the *nicAB* genes in the genomes of different γ- and β-*Proteobacteria*, encodes a TetR-like regulator that represses the activity of *Pa* in the absence of the nicotinic/6-hydroxynicotinic acids as inducers. The nicotinic acid regulatory circuit in *P. putida* has evolved an additional repression loop based on the NicR-dependent cross regulation of the *nicS* gene, thus assuring a tight transcriptional control of the catabolic genes that may prevent depletion of nicotinic acid (vitamin B3) when needed for the synthesis of essential cofactors (Jiménez et al. 2011).

12 Aromatic Compound Responsive Regulators that Belong to Other Families

BadM is a member of the Rrf2 family of transcription factors that acts as a repressor of the *bad* genes involved in the anaerobic degradation of benzoate in *R. palustris*. In vivo data suggest that benzoate- or benzoyl-CoA is the effector for BadM (Hirakawa et al. 2015). Another aromatic compound responsive regulator of the Rrf2 family is the BgeR protein that represses expression of the *bamA* gene encoding the hydrolase for the ring-cleavage step during the anaerobic degradation of benzoate in *Geobacter bemidjiensis*. It was suggested that BgeR plays a key role in regulating the genes involved in the anaerobic degradation of aromatic compounds in *Geobacter* species (Ueki 2011).

The PadR-type regulators contain a wHTH domain with about 80–90 residues that is responsible for the binding to target DNA. The variable C-terminal domain in PadR-like proteins is involved in dimerization through a leucine zipper-like structure. In *Corynebacterium glutamicum* the *van* operon involved in vanillate degradation is regulated by VanR, a PadR-type repressor. VanR forms a dimer and binds cooperatively to two overlapping 24-bp inverted repeats of the target promoter, being vanillate the effector molecule that avoids formation of the protein-DNA complex. It is proposed that VanR-DNA complexes contain two VanR dimers at the VanR operator (Morabbi Heravi et al. 2014).

13 Research Needs

While catabolism of aromatic compounds is relatively well conserved in different organisms, gene regulation shows a wider diversity, and therefore, the whole understanding of the complex regulatory network that controls the expression of the genes involved in a particular degradation pathway is a challenging task. A large number of

aromatic sensing transcriptional regulator sequences have been deposited in databases, but their structure and function remain unknown for most of them. On the other hand, the available metagenomic libraries are a source of still unknown aromatic regulators. Substrate-induced gene expression (SIGEX) is a promoter trap method based on single-cell sorting of clones from a plasmid library using flow cytometry, where transcriptional regulators are identified by the increased expression of a downstream fluorescent reporter gene in the presence, but not in the absence, of an inducing compound. Using SIGEX of a metagenomic library, several transcriptional regulators with different compound specificities and induction rates have been successfully identified (Uchiyama and Miyazaki 2013, Meier et al. 2015). However, SIGEX is limited in several important ways, e.g., distal location of the regulators from the target promoters, library sizes, the substrate of a pathway is not always the cognate inducer, etc., and there is a need for novel methods and strategies for high-throughput screening of aromatic regulators.

A structural understanding of effector binding to a regulatory protein and the molecular mechanisms by which ligands affect derepression/activation at the target promoter is critical. Recent advances in identifying ligand-binding pockets in some regulators may furnish a tool toward identifying the ligands for homologous regulators for which the effector remains unknown. The biological role of antagonists modulating the effect of the agonists (effectors) on the cognate regulators when bacteria are exposed to complex mixtures of aromatic substrates should be also addressed. The characterization of novel modulators that fine-tune the activation of the specific regulators, e.g., by preventing gratuitous induction, requires further studies. An obvious question still unanswered is why the regulatory proteins have evolved so divergently despite regulating very similar pathways for degradation of similar compounds.

The complex regulatory network underlying the hierarchical use of aromatic compounds when bacteria grow in mixtures of these carbon sources in the environment needs to be unraveled. Moreover, the ecophysiological meaning of the diversity found in the regulation of the hierarchical utilization of aromatic compounds among closely related strains sharing ecological niches should be addressed. In this sense, a more complete view of the molecular mechanisms underlying carbon catabolite repression and, in general, other ways of global regulation that sense the physiological status of the cell and overtake the effector-specific regulation of a particular aromatic catabolic pathway should be explored further. Then, computational tools should be used to study the logic structure of the intricate regulatory networks and to formalize it as a digital circuit by converting all known molecular interactions into binary logic operations (logicome) (Silva-Rocha et al. 2011). The integration of future regulatory models with the current genome-scale metabolic models should be a further step for a more accurate in silico reconstruction of bacterial metabolism. On the other hand, it is currently known that microbial populations exploit metabolic diversification of single cells to achieve phenotypic diversity and survive to unpredictable adverse changes in environmental conditions. Studying the regulatory circuits that drive gene expression in individual cells is, therefore, warranted.

From a biotechnology point of view, the in vitro evolution or de novo synthesis of new regulators exhibiting novel specificities and effector-binding affinities is an interesting way to track the evolutionary roadmap of these proteins and to engineer new synthetic regulatory circuits or to develop genetic traps to survey new enzymatic activities in metagenomic libraries.

In summary, a deeper understanding of the complex regulatory network that controls aromatic metabolism will pave the way for the forward engineering of bacteria as efficient biocatalysts for bioremediation of chemical waste and/or biotransformation to biofuels and renewable chemicals, for detection of toxic molecules (biosensors), and for biomedical applications.

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References

Agari Y, Kuramitsu S, Shinkai A (2010) Identification of novel genes regulated by the oxidative stress-responsive transcriptional activator SdrP in *Thermus thermophilus* HB8. FEMS Microbiol Lett 313:127–134

Agari Y, Sakamoto K, Kuramitsu S, Shinkai A (2012) Transcriptional repression mediated by a TetR family protein, PfmR, from *Thermus thermophilus* HB8. J Bacteriol 194:4630–4641

Arai H, Chang MY, Kudo T, Ohishi T (2000) Arrangement and regulation of the genes for *meta*-pathway enzymes required for degradation of phenol in *Comamonas testosteroni* TA441. Microbiology 146:1707–1715

Arai H, Kudo T, Yamamoto T, Ohishi T, Shimizu T, Nakata T (1999) Genetic organization and characteristics of the 3-(3-hydroxyphenyl)propionic acid degradation pathway of *Comamonas testosteroni* TA441. Microbiology 145:2813–2820

Arenghi FL, Pinti M, Galli E, Barbieri P (1999) Identification of the *Pseudomonas stutzeri* OX1 toluene-*o*-xylene monooxygenase regulatory gene (*touR*) and of its cognate promoter. Appl Environ Microbiol 65:4057–4063

Arias-Barrau E, Olivera ERR, Luengo JMM, Fernández C, Galán B, García JL, Díaz E, Miñambres B (2004) The homogentisate pathway: a central catabolic pathway involved in the degradation of L-phenylalanine, L-tyrosine, and 3-hydroxyphenylacetate in *Pseudomonas putida*. J Bacteriol 186:5062–5077

Barragán MJL, Blázquez B, Zamarro MT, Mancheño JM, Jl G, Díaz E, Carmona M (2005) BzdR, a repressor that controls the anaerobic catabolism of benzoate in *Azoarcus* sp. CIB, is the first member of a new subfamily of transcriptional regulators. J Biol Chem 280: 10683–10694

Beck LL, Smith TG, Hoover TR (2007) Look, no hands! Unconventional transcriptional activators in bacteria. Trends Microbiol 15:530–537

Bleichrodt FS, Fischer R, Gerischer UC (2010) The beta-ketoadipate pathway of *Acinetobacter baylyi* undergoes carbon catabolite repression, cross-regulation and vertical regulation, and is affected by Crc. Microbiology 156:1313–1322

Breinig S, Schiltz E, Fuchs G (2000) Genes involved in anaerobic metabolism of phenol in the bacterium *Thauera aromatica*. J Bacteriol 182:5849–5863

Brzostowicz PC, Reams AB, Clark TJ, Neidle EL (2003) Transcriptional cross-regulation of the catechol and protocatechuate branches of the β-ketoadipate pathway contributes to carbon

source-dependent expression of the *Acinetobacter* sp. strain ADP1 *pobA* gene. Appl Environ Microbiol 69:1598–1606

Busby S, Ebright RH (1999) Transcription activation by catabolite activator protein (CAP). J Mol Biol 293:199–213

Bush M, Dixon R (2012) The role of bacterial enhancer binding proteins as specialized activators of σ54-dependent transcription. Microbiol Mol Biol Rev 76:497–529

Büsing I, Kant M, Dörries M, Wöhlbrand L, Rabus R (2015) The predicted σ54-dependent regulator EtpR is essential for expression of genes for anaerobic *p*-ethylphenol and *p*-hydroxyacetophenone degradation in "*Aromatoleum aromaticum*" EbN1. BMC Microbiol 15:251

Byrne AM, Olsen RH (1996) Cascade regulation of the toluene-3-monooxygenase operon (*tbuA1UBVA2C*) of *Burkholderia pickettii* PKO1: role of the *tbuA1* promoter (*PtbuA1*) in the expression of its cognate activator, TbuT. J Bacteriol 178:6327–6337

Calisti C, Ficca AG, Barghini P, Ruzzi M (2008) Regulation of ferulic catabolic genes in *Pseudomonas fluorescens* BF13: involvement of a MarR family regulator. Appl Microbiol Biotechnol 80:475–483

Calles B, de Lorenzo V (2013) Expanding the boolean logic of the prokaryotic transcription factor XylR by functionalization of permissive sites with a protease-target sequence. ACS Synth Biol 2:594–603

Carl B, Fetzner S (2005) Transcriptional activation of quinoline degradation operons of *Pseudomonas putida* 86 by the AraC/XylS-type regulator OxoS and cross-regulation of the PqorM promoter by XylS. Appl Environ Microbiol 71:8618–8626

Carmona M, Prieto MA, Galán B, García JL, Díaz E (2008) Signaling networks and design of pollutants biosensors. In: Díaz E (ed) Microbial Biodegradation: genomics and molecular biology. Caister Academic Press, Norfolk, pp 97–143

Cases I, de Lorenzo V (2005) Promoters in the environment: transcriptional regulation in its natural context. Nat Rev Microbiol 3:105–118

Chao H, Zhou N (2014) Involvement of the global regulator GlxR in 3-hydroxybenzoate and gentisate utilization by *Corynebacterium glutamicum*. Appl Environ Microbiol 80:4215–4225

Chao H, Zhou N (2013) GenR, an IclR-type regulator, activates and represses the transcription of genes involved in 3-hydroxybenzoate and gentisate catabolism in *Corynebacterium glutamicum*. J Bacteriol 195:1598–1609

Chen DW, Zhang Y, Jiang CY, Liu SJ (2014) Benzoate metabolism intermediate benzoyl-coenzyme a affects gentisate pathway regulation in *Comamonas testosteroni*. Appl Environ Microbiol 80:4051–4062

Chen X, Kohl TA, Ruckert C, Rodionov DA, Li LH, Ding JY, Kalinowski J, Liu SJ (2012) Phenylacetic acid catabolism and its transcriptional regulation in *Corynebacterium glutamicum*. Appl Environ Microbiol 78:5796–5804

Cheng M, Chen K, Guo S, Huang X, He J, Li SJJ (2015) PbaR, an IclR family transcriptional activator for the regulation of the 3-phenoxybenzoate 1′,2′-dioxygenase gene cluster in *Sphingobium wenxiniae* JZ-1T. Appl Environ Microbiol 81:8094–8092

Choi KY, Kang BS, Nam MH, Sul WJ, Kim E (2015) Functional identification of OphR, an IclR family transcriptional regulator involved in the regulation of the phthalate catabolic operon in *Rhodococcus* sp. strain DK17. Indian J Microbiol 55:313–318

Collier LS, Gaines GL, Neidle EL (1998) Regulation of benzoate degradation in *Acinetobacter* sp. strain ADP1 by BenM, a LysR-type transcriptional activator. J Bacteriol 180:2493–2501

Cowles CE, Nichols NN, Harwood CS (2000) BenR, a XylS homologue, regulates three different pathways of aromatic acid degradation in *Pseudomonas putida*. J Bacteriol 182:6339–6346

Davis JR, Brown BL, Page R, Sello JK (2013) Study of PcaV from *Streptomyces coelicolor* yields new insights into ligand-responsive MarR family transcription factors. Nucleic Acids Res 41: 3888–3900

de las Heras A, Chavarría M, de Lorenzo V (2011) Association of *dnt* genes of *Burkholderia* sp. DNT with the substrate-blind regulator DntR draws the evolutionary itinerary of 2,4-dinitrotoluene biodegradation. Mol Microbiol 82:287–299

de las Heras A, de Lorenzo V (2011) Cooperative amino acid changes shift the response of the σ54-dependent regulator XylR from natural *m*-xylene towards xenobiotic 2,4-dinitrotoluene. Mol Microbiol 79:1248–1259

del Peso-Santos T, Shingler V (2016) Inter-sigmulon communication through topological promoter coupling. Nucleic Acids Res 44:9638–9649

del Peso-Santos T, Bartolome-Martín D, Fernández C, Alonso S, García JL, Díaz E, Shingler V, Perera J (2006) Coregulation by phenylacetyl-coenzyme a-responsive PaaX integrates control of the upper and lower pathways for catabolism of styrene by *Pseudomonas* sp. strain Y2. J Bacteriol 188:4812–4821

del Peso-Santos T, Bernardo LMD, Skarfstad E, Holmfeldt L, Togneri P, Shingler V (2011) A hyper-mutant of the unusual σ70-*Pr* promoter bypasses synergistic ppGpp/DksA co-stimulation. Nucleic Acids Res 39:5853–5865

Díaz E, Ferrández A, García JL (1998) Characterization of the *hca* cluster encoding the dioxygenolytic pathway for initial catabolism of 3-phenylpropionic acid in *Escherichia coli* K-12. J Bacteriol 180:2915–2923

Díaz E, Jiménez JI, Nogales J (2013) Aerobic degradation of aromatic compounds. Curr Opin Biotechnol 24:431–442

Díaz E, Prieto MA (2000) Bacterial promoters triggering biodegradation of aromatic pollutants. Curr Opin Biotechnol 11:467–475

Díaz E, Ferrández A, Prieto MA, García JL (2001) Biodegradation of aromatic compounds by Escherichia coli. Microbiol Mol Biol Rev 65:523–569

DiMarco AA, Ornston LN (1994) Regulation of *p*-hydroxybenzoate hydroxylase synthesis by PobR bound to an operator in *Acinetobacter calcoaceticus*. J Bacteriol 176:4277–4284

Dispensa M, Thomas CT, Kim MK, Perrotta JA, Gibson J, Harwood CS (1992) Anaerobic growth of *Rhodopseudomonas palustris* on 4-hydroxybenzoate is dependent on AadR, a member of the cyclic AMP receptor protein family of transcriptional regulators. J Bacteriol 174:5803–5813

Domínguez-Cuevas P, González-Pastor JE, Marqués S, Ramos JL, de Lorenzo V (2006) Transcriptional tradeoff between metabolic and stress-response programs in *Pseudomonas putida* KT2440 cells exposed to toluene. J Biol Chem 281:11981–11991

Domínguez-Cuevas P, Marín P, Busby S, Ramos JL, Marqués S (2008) Roles of effectors in XylS-dependent transcription activation: intramolecular domain derepression and DNA binding. J Bacteriol 190:3118–3128

Domínguez-Cuevas P, Ramos JL, Marqués S (2010) Sequential XylS-CTD binding to the *Pm* promoter induces DNA bending prior to activation. J Bacteriol 192:2682–2690

Donoso RA, Pérez-Pantoja D, González B (2011) Strict and direct transcriptional repression of the *pobA* gene by benzoate avoids 4-hydroxybenzoate degradation in the pollutant degrader bacterium *Cupriavidus necator* JMP134. Environ Microbiol 13:1590–1600

Durante-Rodríguez G, Mancheño JM, Díaz E, Carmona M (2016) Refactoring the λ phage lytic/lysogenic decision with a synthetic regulator. Microbiol Open 5:575–581

Durante-Rodríguez G, Mancheño JM, Rivas G, Alfonso C, García JL, Díaz E, Carmona M (2013) Identification of a missing link in the evolution of an enzyme into a transcriptional regulator. PLoS One 8:e57518

Durante-Rodríguez G, Valderrama JA, Mancheño JM, Rivas G, Alfonso C, Arias-Palomo E, Llorca O, García JL, Díaz E, Carmona M (2010) Biochemical characterization of the transcriptional regulator BzdR from *Azoarcus* sp. CIB J Biol Chem 285:35694–35705

Durante-Rodríguez G, Zamarro MT, García JL, Díaz E, Carmona M (2006) Oxygen-dependent regulation of the central pathway for the anaerobic catabolism of aromatic compounds in *Azoarcus* sp. strain CIB. J Bacteriol 188:2343–2354

Eaton RW (1997) *p*-cymene catabolic pathway in *Pseudomonas putida* F1: cloning and characterization of DNA encoding conversion of *p*-cymene to *p*-cumate. J Bacteriol 179:3171–3180

Egland PG, Harwood CS (2000) HbaR, a 4-hydroxybenzoate sensor and FNR-CRP superfamily member, regulates anaerobic 4-hydroxybenzoate degradation by *Rhodopseudomonas palustris*. J Bacteriol 182:100–106

Ezezika OC, Haddad S, Clark TJ, Neidle EL, Momany C (2007) Distinct effector-binding sites enable synergistic transcriptional activation by BenM, a LysR-type regulator. J Mol Biol 367: 616–629

Fernández C, Díaz E, García JL (2013) Insights on the regulation of the phenylacetate degradation pathway from *Escherichia coli*. Environ Microbiol Rep 6:239–250

Ferrández A, García JL, Díaz E (2000) Transcriptional regulation of the divergent *paa* catabolic operons for phenylacetic acid degradation in *Escherichia coli*. J Biol Chem 275:12214–12222

Fujihara H, Yoshida H, Matsunaga T, Goto M, Furukawa K (2006) Cross-regulation of biphenyl- and salicylate-catabolic genes by two regulatory systems in *Pseudomonas pseudoalcaligenes* KF707. J Bacteriol 188:4690–4697

Fukuhara Y, Inakazu K, Kodama N, Kamimura N, Kasai D, Katayama Y, Fukuda M, Masai E (2009) Characterization of the isophthalate degradation genes of *Comamonas* sp. strain E6. Appl Environ Microbiol 76:519–527

Gabor K, Hailesellasse Sene K, Smidt H, de Vos WM, van der Oost J (2008) Divergent roles of CprK paralogues from *Desulfitobacterium hafniense* in activating gene expression. Microbiology 154:3686–3696

Galán B, García JL, Prieto MA (2004) The PaaX repressor, a link between penicillin G acylase and the phenylacetyl-coenzyme A catabolon of *Escherichia coli* W. J Bacteriol 186: 2215–2220

Galán B, Kolb A, Sanz J, García JL, Prieto MA (2003) Molecular determinants of the *hpa* regulatory system of *Escherichia coli*: the HpaR repressor. Nucleic Acids Res 31:6598–6609

Gallegos MT, Michán C, Ramos JL (1993) The XylS/AraC family of regulators. Nucleic Acids Res 21:807–810

Galvão TC, de Lorenzo V (2006) Transcriptional regulators *à la carte*: engineering new effector specificities in bacterial regulatory proteins. Curr Opin Biotechnol 17:34–42

García B, Olivera ER, Minambres B, Carnicero D, Muniz C, Naharro G, Luengo JM (2000) Phenylacetyl-coenzyme a is the true inducer of the phenylacetic acid catabolism pathway in *Pseudomonas putida* U. Appl Environ Microbiol 66:4575–4578

García LL, Rivas-Marín E, Floriano B, Bernhardt R, Ewen KM, Reyes-Ramírez F, Santero E (2011) ThnY is a ferredoxin reductase-like iron-sulfur flavoprotein that has evolved to function as a regulator of tetralin biodegradation gene expression. J Biol Chem 286:1709–1718

Garmendia J, de las Heras A, Galvão TC, de Lorenzo V (2008) Tracing explosives in soil with transcriptional regulators of *Pseudomonas putida* evolved for responding to nitrotoluenes. Microb Biotechnol 1:236–246

Garmendia J, de Lorenzo V (2000) The role of the interdomain B linker in the activation of the XylR protein of *Pseudomonas putida*. Mol Microbiol 38:401–410

Gerischer U, Segura A, Ornston LN (1998) PcaU, a transcriptional activator of genes for protocatechuate utilization in *Acinetobacter*. J Bacteriol 180:1512–1524

Grove A (2013) MarR family transcription factors. Curr Biol 23:R142–R143

Guantes R, Benedetti I, Silva-Rocha R, de Lorenzo V (2015) Transcription factor levels enable metabolic diversification of single cells of environmental bacteria. ISME J 10:1122–1133

Gulvik CA, Buchan A (2013) Simultaneous catabolism of plant-derived aromatic compounds results in enhanced growth for members of the *Roseobacter* lineage. Appl Environ Microbiol 79:3716–3723

Guo Z, Houghton JE (1999) PcaR-mediated activation and repression of *pca* genes from *Pseudomonas putida* are propagated by its binding to both the −35 and the −10 promoter elements. Mol Microbiol 32:253–263

Gupta S, Saxena M, Saini N, Mahmooduzzafar KR, Kumar A (2012) An effective strategy for a whole-cell biosensor based on putative effector interaction site of the regulatory DmpR protein. PLoS One 7:e43527

Herrera MC, Duque E, Rodríguez-Herva JJ, Fernández-Escamilla AM, Ramos JL (2010) Identification and characterization of the PhhR regulon in *Pseudomonas putida*. Environ Microbiol 12 (6):1427–1438

Hirakawa H, Hirakawa Y, Greenberg EP, Harwood CS (2015) BadR and BadM proteins transcriptionally regulate two operons needed for anaerobic benzoate degradation by *Rhodopseudomonas palustris*. Appl Environ Microbiol 81:4253–4262

Hirakawa H, Schaefer AL, Greenberg EP, Harwood CS (2012) Anaerobic *p*-coumarate degradation by *Rhodopseudomonas palustris* and identification of CouR, a MarR repressor protein that binds *p*-coumaroyl coenzyme A. J Bacteriol 194:1960–1967

Horbal L, Fedorenko V, Luzhetskyy A (2014) Novel and tightly regulated resorcinol and cumate-inducible expression systems for *Streptomyces* and other actinobacteria. Appl Microbiol Biotechnol 98:8641–8655

Hoskisson PA, Rigali S (2009) Chapter 1: Variation in Form and Function: the helix-turn-helix regulators of the GntR superfamily. Adv Appl Microbiol 69:1–22

Jain D, Nair DT (2012) Spacing between core recognition motifs determines relative orientation of AraR monomers on bipartite operators. Nucleic Acids Res 41:639–647

Jiménez JI, Juárez JF, García JL, Díaz E (2011) A finely tuned regulatory circuit of the nicotinic acid degradation pathway in *Pseudomonas putida*. Environ Microbiol 13:1718–1732

Jõesaar M, Heinaru E, Viggor S, Vedler E, Heinaru A (2010) Diversity of the transcriptional regulation of the *pch* gene cluster in two indigenous *p*-cresol-degradative strains of *Pseudomonas fluorescens*. FEMS Microbiol Ecol 72:464–475

Jones RM, Pagmantidis V, Williams PA (2000) *sal* genes determining the catabolism of salicylate esters are part of a supraoperonic cluster of catabolic genes in *Acinetobacter* sp. strain ADP1. J Bacteriol 182:2018–2025

Jones RM, Williams PA (2001) *areCBA* is an operon in *Acinetobacter* sp. strain ADP1 and is controlled by AreR, a σ54-dependent regulator. J Bacteriol 183:405–409

Jourlin-Castelli C, Mani N, Nakano MM, Sonenshein AL (2000) CcpC, a novel regulator of the LysR family required for glucose repression of the *citB* gene in *Bacillus subtilis*. J Mol Biol 295:865–878

Juárez JF, Liu H, Zamarro MT, McMahon S, Liu H, Naismith JH, Eberlein C, Boll M, Carmona M, Díaz E (2015) Unraveling the specific regulation of the central pathway for anaerobic degradation of 3-methylbenzoate. J Biol Chem 290:12165–12183

Juárez JF, Zamarro MT, Eberlein C, Boll M, Carmona M, Díaz E (2012) Characterization of the *mbd* cluster encoding the anaerobic 3-methylbenzoyl-CoA central pathway. Environ Microbiol 15:148–166

Kamimura N, Takamura K, Hara H, Kasai D, Natsume R, Senda T, Katayama Y, Fukuda M, Masai E (2010) Regulatory system of the protocatechuate 4,5-cleavage pathway genes essential for lignin downstream catabolism. J Bacteriol 192:3394–3405

Kasai D, Araki N, Motoi K, Yoshikawa S, Iino T, Imai S, Masai E, Fukuda M (2015) γ-resorcylate catabolic-pathway genes in the soil Actinomycete *Rhodococcus jostii* RHA1. Appl Environ Microbiol 81:7656–7665

Kasai D, Kamimura N, Tani K, Umeda S, Abe T, Fukuda M, Masai E (2012) Characterization of FerC, a MarR-type transcriptional regulator, involved in transcriptional regulation of the ferulate catabolic operon in *Sphingobium* sp. strain SYK-6. FEMS Microbiol Lett 332:68–75

Kasai D, Kitajima M, Fukuda M, Masai E (2010) Transcriptional regulation of the terephthalate catabolism operon in *Comamonas* sp. strain E6. Appl Environ Microbiol 76:6047–6055

Kim HS, Kang TS, Hyun JS, Kang HS (2004) Regulation of penicillin G acylase gene expression in *Escherichia coli* by repressor PaaX and the cAMP- receptor protein complex. J Biol Chem 279:33253–33262

Kim MN, Park HH, Lim WK, Shin HJ (2005) Construction and comparison of *Escherichia coli* whole-cell biosensors capable of detecting aromatic compounds. J Microbiol Methods 60: 235–245

Kim SI, Jourlin-Castelli C, Wellington SR, Sonenshein AL (2003) Mechanism of repression by *Bacillus subtilis* CcpC, a LysR family regulator. J Mol Biol 334:609–624

Kim Y, Joachimiak G, Bigelow L, Babnigg G, Joachimiak A (2016) How aromatic compounds block DNA binding of HcaR catabolite regulator. J Biol Chem 291:13243–13256

Körner H, Sofia HJ, Zumft WG (2003) Phylogeny of the bacterial superfamily of Crp-Fnr transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs. FEMS Microbiol Rev 27:559–592

Krell T, Molina-Henares AJ, Ramos JL (2006) The IclR family of transcriptional activators and repressors can be defined by a single profile. Protein Sci 15:1207–1213

Leahy JG, Johnson GR, Olsen RH (1997) Cross-regulation of toluene monooxygenases by the transcriptional activators TbmR and TbuT. Appl Environ Microbiol 63:3736–3739

Ledesma-García L, Sánchez-Azqueta A, Medina M, Reyes-Ramírez F, Santero E (2016) Redox proteins of hydroxylating bacterial dioxygenases establish a regulatory cascade that prevents gratuitous induction of tetralin biodegradation genes. Sci Rep 6:23848

Lerche M, Dian C, Round A, Lönneborg R, Brzezinski P, Leonard GA (2016) The solution configurations of inactive and activated DntR have implications for the sliding dimer mechanism of LysR transcription factors. Sci Rep 6:19988

Levy C, Pike K, Heyes DJ, Joyce MG, Gabor K, Smidt H, van der Oost J, Leys D (2008) Molecular basis of halorespiration control by CprK, a CRP-FNR type transcriptional regulator. Mol Microbiol 70:151–167

Li DF, Zhang N, Hou YJ, Huang Y, Hu Y, Zhang Y, Liu SJ, Wang DC (2011) Crystal structures of the transcriptional repressor RolR reveals a novel recognition mechanism between inducer and regulator. PLoS One 6:e19529

Li T, Zhao K, Huang Y, Li D, Jiang CY, Zhou N, Fan Z, Liu SJ (2012) The TetR-type transcriptional repressor RolR from *Corynebacterium glutamicum* regulates resorcinol catabolism by binding to a unique operator, *rolO*. Appl Environ Microbiol 78:6009–6016

Lin GH, Chen HP, Shu HY (2015) Detoxification of indole by an indole-induced flavoprotein oxygenase from *Acinetobacter baumannii*. PLoS One 10:e0138798

López-Sánchez A, Rivas-Marín E, Martínez-Pérez O, Floriano B, Santero E (2009) Co-ordinated regulation of two divergent promoters through higher-order complex formation by the LysR-type regulator ThnR. Mol Microbiol 73:1086–1100

Lovley DR (2003) Cleaning up with genomics: applying molecular biology to bioremediation. Nat Rev Microbiol 1:35–44

Maddocks SE, Oyston PCF (2008) Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. Microbiology 154:3609–3623

Madhushani A, del Peso-Santos T, Moreno R, Rojo F, Shingler V (2014) Transcriptional and translational control through the 5′-leader region of the *dmpR* master regulatory gene of phenol metabolism. Environ Microbiol 17:119–133

Manso I, Torres B, Andreu JM, Menéndez M, Rivas G, Alfonso C, Díaz E, García JL, Galán B (2009) 3-Hydroxyphenylpropionate and phenylpropionate are synergistic activators of the MhpR transcriptional regulator from *Escherichia coli*. J Biol Chem 284:21218–21228

Marqués S, Manzanera M, González-Pérez MM, Gallegos MT, Ramos JL (1999) The XylS-dependent *Pm* promoter is transcribed in vivo by RNA polymerase with σ32 or σ38 depending on the growth phase. Mol Microbiol 31:1105–1113

McFall SM, Chugani SA, Chakrabarty AM (1998) Transcriptional activation of the catechol and chlorocatechol operons: variations on a theme. Gene 223:257–267

Meier MJ, Paterson ES, Lambert IB (2015) Use of substrate-induced gene expression in metagenomic analysis of an aromatic hydrocarbon-contaminated soil. Appl Environ Microbiol 82: 897–909

Michán C, Zhou L, Gallegos MT, Timmis KN, Ramos JL (1992) Identification of critical amino-terminal regions of XylS. The positive regulator encoded by the TOL plasmid. J Biol Chem 267:22897–22901

Miyakoshi M, Urata M, Habe H, Omori T, Yamane H, Nojiri H (2006) Differentiation of carbazole catabolic operons by replacement of the regulated promoter via transposition of an insertion sequence. J Biol Chem 281:8450–8457

Mohamed M, Ismail W, Heider J, Fuchs G (2002) Aerobic metabolism of phenylacetic acids in *Azoarcus evansii*. Arch Microbiol 178:180–192

Molina-Fuentes A, Pacheco D, Marín P, Philipp B, Schink B, Marqués S (2015) Identification of the gene cluster for the anaerobic degradation of 3,5-dihydroxybenzoate (α-resorcylate) in *Thauera aromatica* strain AR-1. Appl Environ Microbiol 81:7201–7214

Molina-Henares AJ, Krell T, Eugenia Guazzaroni M, Segura A, Ramos JL (2006) Members of the IclR family of bacterial transcriptional regulators function as activators and/or repressors. FEMS Microbiol Rev 30:157–186

Monferrer D, Tralau T, Kertesz MA, Dix I, Solà M, Usón I (2010) Structural studies on the full-length LysR-type regulator TsaR from *Comamonas testosteroni* T-2 reveal a novel open conformation of the tetrameric LTTR fold. Mol Microbiol 75:1199–1214

Morabbi Heravi K, Lange J, Watzlawick H, Kalinowski J, Altenbuchner J (2014) Transcriptional regulation of the vanillate utilization genes (*vanABK* operon) of *Corynebacterium glutamicum* by VanR, a PadR-like repressor. J Bacteriol 197:959–972

Morawski B, Segura A, Ornston LN (2000) Repression of *Acinetobacter* vanillate demethylase synthesis by VanR, a member of the GntR family of transcriptional regulators. FEMS Microbiol Lett 187:65–68

Moreno R, Hernández-Arranz S, la Rosa R, Yuste L, Madhushani A, Shingler V, Rojo F (2015) The Crc and Hfq proteins of *Pseudomonas putida* cooperate in catabolite repression and formation of ribonucleic acid complexes with specific target motifs. Environ Microbiol 17:105–118

Mouz S, Merlin C, Springael D (1999) A GntR-like negative regulator of the biphenyl degradation genes of the transposon Tn*4371*. Mol Gen Genet 262:790–799

Muraoka S, Okumura R, Ogawa N, Nonaka T, Miyashita K, Senda T (2003) Crystal structure of a full-length LysR-type transcriptional regulator, CbnR: unusual combination of two subunit forms and molecular bases for causing and changing DNA bend. J Mol Biol 328:555–566

Nga DP, Altenbuchner J, Heiss GS (2004) NpdR, a repressor involved in 2,4,6-trinitrophenol degradation in *Rhodococcus opacus* HL PM-1. J Bacteriol 186:98–103

Niewerth H, Parschat K, Rauschenberg M, Ravoo BJ, Fetzner S (2012) The PaaX-type repressor MeqR2 of *Arthrobacter* sp. strain Rue61a, involved in the regulation of quinaldine catabolism, binds to its own promoter and to catabolic promoters and specifically responds to anthraniloyl coenzyme A. J Bacteriol 195:1068–1080

O'Neill E (2001) An active role for a structured B-linker in effector control of the σ54-dependent regulator DmpR. EMBO J 20:819–827

Ogawa N, McFall SM, Klem TJ, Miyashita K, Chakrabarty AM (1999) Transcriptional activation of the chlorocatechol degradative genes of *Ralstonia eutropha* NH9. J Bacteriol 181:6697–6705

Ohtsubo Y, Delawary M, Kimbara K, Takagi M, Ohta A, Nagata Y (2001) BphS, a key transcriptional regulator of *bph* genes involved in polychlorinated biphenyl/biphenyl degradation in *Pseudomonas* sp. KKS102. J Biol Chem 276:36146–36154

Orth P, Schnappinger D, Hillen W, Saenger W, Hinrichs W (2000) Structural basis of gene regulation by the tetracycline inducible Tet repressor-operator system. Nat Struct Biol 7: 215–219

Otani H, Stogios PJ, Xu X, Nocek B, Li SN, Savchenko A, Eltis LD (2015) The activity of CouR, a MarR family transcriptional regulator, is modulated through a novel molecular mechanism. Nucleic Acids Res 44:595–607

Pareja E, Pareja-Tobes P, Manrique M, Pareja-Tobes E, Bonal J, Tobes R (2006) ExtraTrain: a database of extragenic regions and transcriptional information in prokaryotic organisms. BMC Microbiol 6:29

Park HS, Kim HS (2001) Genetic and structural organization of the aminophenol catabolic operon and its implication for evolutionary process. J Bacteriol 183:5074–5081

Parke D (1996) Characterization of PcaQ, a LysR-type transcriptional activator required for catabolism of phenolic compounds, from *Agrobacterium tumefaciens*. J Bacteriol 178: 266–272

Parke D, Ornston LN (2003) Hydroxycinnamate (*hca*) catabolic genes from *Acinetobacter* sp. strain ADP1 are repressed by *hcaR* and are induced by hydroxycinnamoyl-coenzyme A thioesters. Appl Environ Microbiol 69:5398–5409

Pérez-Martín J, de Lorenzo V (1995) The amino-terminal domain of the prokaryotic enhancer-binding protein XylR is a specific intramolecular repressor. Proc Natl Acad Sci USA 92: 9392–9396

Pérez-Pantoja D, Leiva-Novoa P, Donoso RA, Little C, Godoy M, Pieper DH, González B (2015) Hierarchy of carbon source utilization in soil bacteria: hegemonic preference for benzoate in complex aromatic compound mixtures degraded by *Cupriavidus pinatubonensis* strain JMP134. Appl Environ Microbiol 81:3914–3924

Phattarasukol S, Radey MC, Lappala CR, Oda Y, Hirakawa H, Brittnacher MJ, Harwood CS (2012) Identification of a *p*-coumarate degradation regulon in *Rhodopseudomonas palustris* by Xpression, an integrated tool for prokaryotic RNA-Seq data processing. Appl Environ Microbiol 78:6812–6818

Picossi S, Belitsky BR, Sonenshein AL (2007) Molecular mechanism of the regulation of *Bacillus subtilis gltAB* expression by GltC. J Mol Biol 365:1298–1313

Popp R, Kohl T, Patz P, Trautwein G, Gerischer U (2002) Differential DNA binding of transcriptional regulator PcaU from *Acinetobacter* sp. strain ADP1. J Bacteriol 184:1988–1997

Pop SM, Gupta N, Raza AS, Ragsdale SW (2006) Transcriptional activation of dehalorespiration. Identification of redox-active cysteines regulating dimerization and DNA binding. J Biol Chem 8:26382–26390

Porrúa O, García-Jaramillo M, Santero E, Govantes F (2007) The LysR-type regulator AtzR binding site: DNA sequences involved in activation, repression and cyanuric acid-dependent repositioning. Mol Microbiol 66:410–427

Prieto MA, García JL (1994) Molecular characterization of 4-hydroxyphenylacetate 3-hydroxylase of *Escherichia coli.* A two-protein component enzyme. J Biol Chem 269:22823–22829

Providenti MA, Wyndham RC (2001) Identification and functional characterization of CbaR, a MarR-Like modulator of the *cbaABC*-encoded chlorobenzoate catabolism pathway. Appl Environ Microbiol 67:3530–3541

Quinn JA, McKay DB, Entsch B (2001) Analysis of the *pobA* and *pobR* genes controlling expression of *p*-hydroxybenzoate hydroxylase in *Azotobacter chroococcum*. Gene 264:77–85

Ramos JL, Marqués S, Timmis KN (1997) Transcriptional control of the *Pseudomonas tol* plasmid catabolic operons is achieved through an interplay of host factors and plasmid-encoded regulators. Annu Rev Microbiol 51:341–373

Ramos JL, Martínez-Bueno M, Molina-Henares AJ, Teran W, Watanabe K, Zhang X, Gallegos MT, Brennan R, Tobes R (2005) The TetR family of transcriptional repressors. Microbiol Mol Biol Rev 69:326–356

Rey FE, Harwood CS (2010) FixK, a global regulator of microaerobic growth, controls photosynthesis in *Rhodopseudomonas palustris*. Mol Microbiol 75:1007–1020

Rojas-Altuve A, Carrasco-López C, Hernández-Rocamora VM, Sanz JM, Hermoso JA (2011) Crystallization and preliminary X-ray diffraction studies of the transcriptional repressor PaaX, the main regulator of the phenylacetic acid degradation pathway in *Escherichia coli* W. Acta Cryst Sect F 67:1278–1280

Sakamoto K, Agari Y, Kuramitsu S, Shinkai A (2011) Phenylacetyl coenzyme A is an effector molecule of the TetR family transcriptional repressor PaaR from *Thermus thermophilus* HB8. J Bacteriol 193:4388–4395

Sandu C, Chiribau CB, Brandsch R (2003) Characterization of HdnoR, the transcriptional repressor of the 6-hydroxy-D-nicotine oxidase gene of *Arthrobacter nicotinovorans* pAO1, and its DNA-binding activity in response to L- and D-nicotine derivatives. J Biol Chem 278:51307–51315

Sasson V, Shachrai I, Bren A, Dekel E, Alon U (2012) Mode of regulation and the insulation of bacterial gene expression. Mol Cell 46:399–407

Schell MA (1993) Molecular biology of the LysR family of transcriptional regulators. Annu Rev Microbiol 47:597–626

Seedorff J, Schleif R (2011) Active role of the interdomain linker of AraC. J Bacteriol 193: 5737–5746

Shingler V (2004) Transcriptional regulation and catabolic strategies of phenol degradative pathways. In: Ramos JL (ed) *Pseudomonas*, vol 2. Kluwer Academic, New York, pp 451–477

Shu HY, Lin LC, Lin TK, Chen HP, Yang HH, Peng KC, Lin GH (2015) Transcriptional regulation of the *iac* locus from *Acinetobacter baumannii* by the phytohormone indole-3-acetic acid. Antonie Van Leeuwenhoek 107:1237–1247

Silva-Jiménez H, García-Fontana C, Cadirci BH, Ramos-González MI, Ramos JL, Krell T (2011) Study of the TmoS/TmoT two-component system: towards the functional characterization of the family of TodS/TodT like systems. Microb Biotechnol 5:489–500

Silva-Rocha R, de Jong H, Tamames J, de Lorenzo V (2011) The logic layout of the TOL network of *Pseudomonas putida* pWW0 plasmid stems from a metabolic amplifier motif (MAM) that optimizes biodegradation of *m*-xylene. BMC Syst Biol 5:191

Silva-Rocha R, de Lorenzo V (2012) Broadening the signal specificity of prokaryotic promoters by modifying *cis*-regulatory elements associated with a single transcription factor. Mol BioSyst 8:1950

Skarfstad E, O'Neill E, Garmendia J, Shingler V (2000) Identification of an effector specificity subregion within the aromatic-responsive regulators DmpR and XylR by DNA shuffling. J Bacteriol 182:3008–3016

Smirnova IA, Dian C, Leonard GA, McSweeney S, Birse D, Brzezinski P (2004) Development of a bacterial biosensor for nitrotoluenes: the crystal structure of the transcriptional regulator DntR. J Mol Biol 340:405–418

Solera D, Arenghi FLG, Woelk T, Galli E, Barbieri P (2004) TouR-mediated effector-independent growth phase-dependent activation of the σ54 *Ptou* promoter of *Pseudomonas stutzeri* OX1. J Bacteriol 186:7353–7363

Suresh PS, Kumar R, Kumar A (2010) Three dimensional model for N-terminal A domain of DmpR (2-dimethylphenol) protein based on secondary structure prediction and fold recognition. In Silico Biol 10:223–233

Suvorova IA, Korostelev YD, Gelfand MS (2015) GntR family of bacterial transcription factors and their DNA binding motifs: structure, positioning and co-evolution. PLoS One 10:e0132618

Szőköl J, Rucká L, Šimčíková M, Halada P, Nešvera J, Pátek M (2014) Induction and carbon catabolite repression of phenol degradation genes in *Rhodococcus erythropolis* and *Rhodococcus jostii*. Appl Microbiol Biotechnol 98:8267–8279

Teramoto M, Harayama S, Watanabe K (2001) PhcS represses gratuitous expression of phenol-metabolizing enzymes in *Comamonas testosteroni* R5. J Bacteriol 183:4227–4234

Teufel R, Friedrich T, Fuchs G (2012) An oxygenase that forms and deoxygenates toxic epoxide. Nature 483:359–362

Thanbichler M, Iniesta AA, Shapiro L (2007) A comprehensive set of plasmids for vanillate- and xylose-inducible gene expression in *Caulobacter crescentus*. Nucleic Acids Res 35:e137–e137

Tomás-Gallardo L, Santero E, Floriano B (2012) Involvement of a putative cyclic AMP receptor protein (CRP)-like binding sequence and a CRP-like protein in glucose-mediated catabolite repression of *thn* genes in *Rhodococcus* sp. strain TFB. Appl Environ Microbiol 78:5460–5462

Torres B, Porras G, García JL, Díaz E (2003) Regulation of the *mhp* cluster responsible for 3-(3-hydroxyphenyl)propionic acid degradation in *Escherichia coli*. J Biol Chem 278:27575–27585

Townsend PD, Jungwirth B, Pojer F, Bußmann M, Money VA, Cole ST, Pühler A, Tauch A, Bott M, Cann MJ, Pohl E (2014) The crystal structures of Apo and cAMP-bound GlxR from *Corynebacterium glutamicum* reveal structural and dynamic changes upon cAMP binding in CRP/FNR family transcription factors. PLoS One 9:e113265

Tralau T, Cook AM, Ruff J (2003a) An additional regulator, TsaQ, is involved with TsaR in regulation of transport during the degradation of *p*-toluenesulfonate in *Comamonas testosteroni* T-2. Arch Microbiol 180:319–326

Tralau T, Mampel J, Cook AM, Ruff J (2003b) Characterization of TsaR, an oxygen-sensitive LysR-type regulator for the degradation of *p*-toluenesulfonate in *Comamonas testosteroni* T-2. Appl Environ Microbiol 69:2298–2305

Trautwein G, Gerischer U (2001) Effects exerted by tanscriptional regulator PcaU from Acinetobacter sp. strain ADP1. J Bacteriol 183:873–881

Tropel D, van der Meer JR (2004) Bacterial transcriptional regulators for degradation pathways of aromatic compounds. Microbiol Mol Biol Rev 68:474–500

Uchiyama T, Miyazaki K (2013) Metagenomic screening for aromatic compound-responsive transcriptional regulators. PLoS One 8:e75795

Ueki T (2011) Identification of a transcriptional repressor involved in benzoate metabolism in *Geobacter bemidjiensis*. Appl Environ Microbiol 77:7058–7062

Ulrich LE, Koonin EV, Zhulin IB (2005) One-component systems dominate signal transduction in prokaryotes. Trends Microbiol 13:52–56

Urata M, Miyakoshi M, Kai S, Maeda K, Habe H, Omori T, Yamane H, Nojiri H (2004) Transcriptional regulation of the *ant* operon, encoding two-component anthranilate 1,2-dioxygenase, on the carbazole-degradative plasmid pCAR1 of *Pseudomonas resinovorans* strain CA10. J Bacteriol 186:6815–6823

Valderrama JA, Durante-Rodríguez G, Blázquez B, García JL, Carmona M, Díaz E (2012) Bacterial degradation of benzoate: cross-regulation between aerobic and anaerobic pathways. J Biol Chem 287:10494–10508

van Aalten DMF (2001) The structural basis of acyl coenzyme A-dependent regulation of the transcription factor FadR. EMBO J 20:2041–2050

Vaneechoutte M, Young DM, Ornston LN, de Baere T, Nemec A, Van Der Reijden T, Carr E, Tjernberg I, Dijkshoorn L (2006) Naturally transformable *Acinetobacter* sp. strain ADP1 belongs to the newly described species *Acinetobacter baylyi*. Appl Environ Microbiol 72:932–936

Veselý M, Knoppová M, Nešvera J, Pátek M (2007) Analysis of *catRABC* operon for catechol degradation from phenol-degrading *Rhodococcus erythropolis*. Appl Microbiol Biotechnol 76:159–168

Wöhlbrand L, Kallerhoff B, Lange D, Hufnagel P, Thiermann J, Reinhardt R, Rabus R (2007) Functional proteomic view of metabolic regulation in "*Aromatoleum aromaticum*" strain EbN1. Proteomics 7:2222–2239

Xu Y, Heath RJ, Li Z, Rock CO, White SW (2001) The FadR.DNA complex: transcriptional control of fatty acid metabolism in *Escherichia coli*. J Biol Chem 276:17373–17379

Yamamoto K, Ishihama A (2002) Two different modes of transcription repression of the *Escherichia coli* acetate operon by IclR. Mol Microbiol 47:183–194

Yudistira H, McClarty L, Bloodworth RAM, Hammond SA, Butcher H, Mark BL, Cardona ST (2011) Phenylalanine induces *Burkholderia cenocepacia* phenylacetic acid catabolism through degradation to phenylacetyl-CoA in synthetic cystic fibrosis sputum medium. Microb Pathog 51:186–193

Zhao Y, Feng R, Zheng G, Tian J, Ruan L, Ge M, Jiang W, Lu Y (2015) Involvement of the TetR-type regulator PaaR in the regulation of pristinamycin I biosynthesis through an effect on precursor supply in *Streptomyces pristinaespiralis*. J Bacteriol 197:2062–2071