Heay Metal Resistance in Pseudomonads

CHAPTER · DECEMBER 2009

DOI: 10.1007/978-90-481-3909-5_9

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Chapter 9
Heavy Metal Resistance in Pseudomonads

Esther Aguilar-Barajas, Martha I. Ramírez-Díaz, Héctor Riveros-Rosas, and Carlos Cervantes

9.1 Introduction

The metabolic diversity of the genus Pseudomonas (and related bacterial species, called collectively pseudomonads) has attracted researchers to study this versatile microbial group. The ability to thrive in hostile environments, aided by a notable capacity to degrade or tolerate a wide variety of natural and synthetic compounds, results from the possession of highly adapted genomes. About 25 genomes from pseudomonad strains have been sequenced to date, representing eight different species from varied habitats. Genomic analyses conÐrm that pseudomonads evolved complex enzymatic strategies, delicate genetic regulatory switches, and efÐcient transport systems, to keep pace in ever-changing environments. These adaptive mechanisms include those conferring resistance to toxic compounds such as antibiotics and the ions derived from heavy metals and metalloids. Transport systems able to actively efÑux metal ions out from the cytoplasm or the periplasmic space are a key strategy to withstand heavy metal toxicity. As with other bacteria, heavy metal resistance genes in pseudomonads may reside either in the chromosome or within plasmids.

In this chapter, the information concerning the strategies used by pseudomonads to tolerate heavy metals is summarized. It should be noted that some of these resistance mechanisms have been assigned to pseudomonads only by the Ðnding of homologous genes and operons when compared with characterized genes from sequenced genomes of different bacteria. In other cases, biochemical evidence for speciÐc heavy metal resistance systems has been directly provided by the analysis of genes from pseudomonads.

A Ðrst compilation on heavy metal resistance mechanisms in pseudomonads appeared almost two decades ago [1], but an overwhelming amount of information has accumulated since, notably by the advent of the genomic era. More recently, a monograph book covered the interactions of heavy metals with the wider microbial

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J.L. Ramos, A. Filloux (eds.), Pseudomonas, DOI 10.1007/978-90-481-3909-5_9,

world [2]; reference to speciÞc chapters will be given below for studies related to pseudomonads.

For this review, the heavy metal protagonists have been divided into three main groups: (i) micronutrient cations (copper, cobalt, nickel and zinc), (ii) toxic cations (cadmium, lead and mercury), and (iii) toxic oxyanions (arsenate/arsenite, chromate and tellurite). A Þnal recount includes other less-studied toxic ions (silver, tin, selenium) for which some information exists in pseudomonads. For each case, a brief account on the metal(loid) toxicity mechanisms is followed by a description of the resistance strategies reported in pseudomonads or (when missing) the best-studied systems uncovered in related bacteria. A scrutiny of the genomes of Pseudomonas aeruginosa [3, 4] and Pseudomonas putida [5, 6] already showed the presence of numerous metal resistance determinants, including members of the main transporter families: resistance-nodulation-cell division (RND), cation diffusion facilitator (CDF), major facilitator superfamily (MFS), and P-type ATPases able to efÑux toxic metal cations or oxyanions. Regulatory systems for bacterial heavy metal resistance, as for other adaptive strategies, are of paramount importance for cell economy. Expression of the corresponding genetic determinants is subjected to delicate control mechanisms, commonly acting at the transcriptional level [7; reviewed in 8]. These regulatory systems will be mentioned in this review but, for space reasons, will not be detailed.

9.2 Micronutrient Cations

As most living organisms, pseudomonads require the essential micronutrient cations derived from copper, cobalt, nickel and zinc, used mainly as enzyme cofactors and regulatory effectors. For these purposes, divalent cations form complexes with diverse ligands within the cells. Higher concentrations of these transition metals, however, may exert toxic effects on most cells as harmful complexes may be formed with varied biomolecules. This dual behavior has made it necessary for bacteria to develop strict homeostasis mechanisms in order to avoid metal toxicity, while allowing intracellular basal levels of the essential ions. Homeostasis commonly includes transmembrane uptake and efÑux systems that carefully regulate intracellular cation levels. This review emphasizes on those pseudomonad systems devoted to tolerate the noxious effects of toxic divalent cations, not considering the physiological mechanisms for micronutrient acquisition and use. Also, systems for the homeostasis of essential but almost not toxic cations (i.e. iron and manganese) will not be considered.

9.2.1 Copper

Copper is an essential metal, mainly required by aerobic cells as a cofactor for electron transport and redox enzyme systems [9]. Copper exists in the cytoplasm in the Cu(I) reduced state, being its ability to undergo redox Cu(II) to Cu(I) transformations partly responsible of its toxic properties. Additional toxicity effects derive

from the ability of copper to displace other metals (i.e. Ni^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}) from essential complexes as well as to unspeciÞcally bind to biomolecules [10]. Bacterial copper transport and homeostasis has been widely studied in Escherichia coli and in Gram-positive Enterococcus hirae (reviewed in [9, 11Ã15]) and will not be treated here. Some copper resistance systems related to pseudomonads will be next described.

The copABCD operon from Pseudomonas syringae plasmid pPT23D was one of the Þrst bacterial copper-resistance systems analyzed [16]. pPT23D was found in a copper-resistant P. syringae pv. tomato strain isolated from copper-treated tomato Þelds [17]. Unlike other cation resistance mechanisms, the cop operon encodes a copper-sequestering system that prevents copper ions from entering the cytoplasm (Fig. 9.1A). CopA and CopC are periplasmic copper-binding proteins able to capture 11 and 1 copper atoms per polypeptide, respectively [18]. CopA also displays

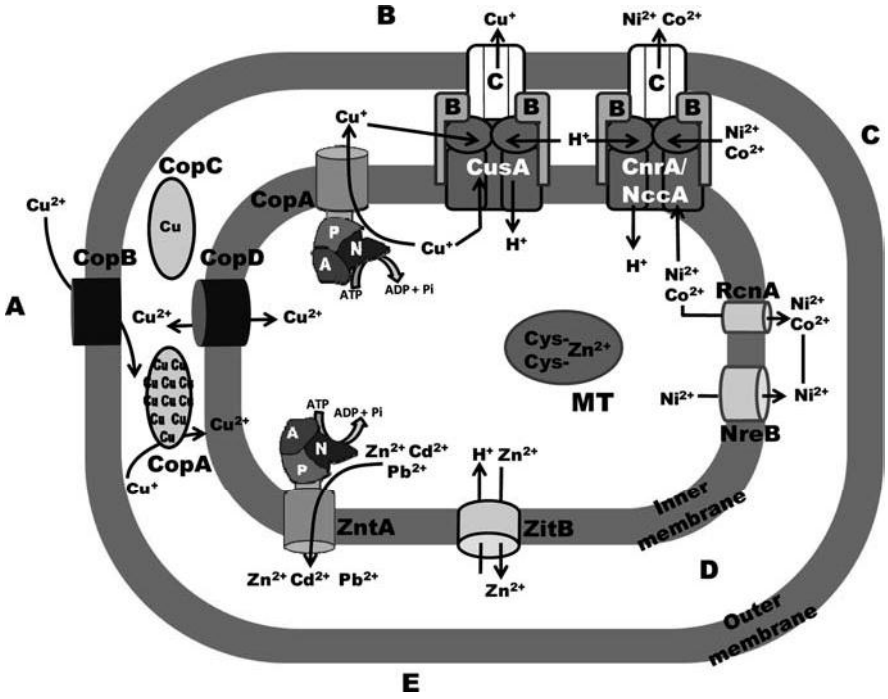

Fig. 9.1 Mechanisms of resistance to essential cations in pseudomonads. A, Cop copper binding system. B, P-type ATPase CopA and RND complex CusCBA. C, nickel/cobalt resistance systems. RND complexes CnrCBA and NccCBA; MFS transporters RcnA and NreB. D, zinc metallothioneins (MT). E, zinc resistance systems. P-type ATPase ZntA and CDF transporter ZitB. P-type ATPases domains shown are: P, phosphorylation domain; N, nucleotide-binding domain; A, activator and phosphatase domain. The functions of the resistance systems are described in the text. Note that CopA in A is a periplasmic multi-copper oxidase of the COG2132 protein family whereas CopA in B is a P-type ATPase of the COG2217 protein family. These two non-homologous proteins received the same name

a multi-copper oxidase activity, transforming Cu(I) to Cu(II), similar to that of CueO from E. coli [19] and may protect periplasmic enzymes from copper damage. CopC is probably a chaperone which delivers copper to CopD, an inner membrane protein with eight transmembrane segments. Outer membrane protein CopB also binds Cu^{2+} and is proposed to function in concert with CopD in Cu^{2+} uptake [20]. Copper-inducible expression of the cop operon is regulated by a chromosomally-encoded repressor and by a plasmid-borne two-component CopR/S system [21]. Chromosomal homologs of CopA and CopB have been identified in many pseudomonads [22], whereas CopC and CopD are less common and seem to be auxiliary determinants for optimal copper resistance. An additional gene, transcribed from a different promoter, encodes the small periplasmic CopE protein, which is related to PcoE from E. coli and to SilE from the Salmonella silver-resistance operon; as PcoE, CopE seems to bind Cu(I) and may function as a copper chaperone [9]. The other pseudomonad cop genes also show sequence similarity with the corresponding genes from the plasmid-mediated E. coli copper-resistance pco operon, although the latter system catalyzes the efflux of copper rather than its binding [23].

Other potential copper-resistant determinants studied in enterobacteria have been identified from the sequenced genomes of pseudomonads. For example, the genomes of P. aeruginosa [3] and P. putida [6] possess homologs of the CusCBA system, a proton-driven RND transporter which effluxes Cu^{+} (and Ag^{+}) from the cytoplasm [24] (Fig. 9.1B), and of SilP, a P-type ATPase which extrudes Ag^{+} (and probably Cu^{+}) [25]. Also, a homolog of the widespread P-type ATPase CopA [26], able to efflux copper from the cytoplasm, has been located in the P. aeruginosa genome [27] (Fig. 9.1B). A transcriptomics analysis of P. aeruginosa PAO1 showed that a P-type ATPase (ORF PA3920), three RND transporters (PA1436, PA2520, and PA3522), and two CDF family members (PA0397 and PA1297) were up-regulated in response to copper exposure [28]. One of the RND determinants encodes the czrCBA system [29], mentioned below because it confers Cd^{2+}, Zn^{2+} and Co^{2+} resistance by an efflux mechanism. The CDF systems encode homologs of the CzcD and RzcB transporters which confer resistance to divalent cations in other bacteria [30]. It is possible that some of these systems also efflux copper. These findings confirm that efflux systems constitute a major strategy for copper homeostasis as well as a main protection barrier for pseudomonads against copper toxicity.

9.2.2 Cobalt and Nickel

Cobalt and nickel are similar transition metals of oxidation state II. They play essential roles for microorganisms as cofactors for many diverse metalloenzymes. Thus, uptake and homeostasis systems for these micronutrient divalent cations must exist in all bacteria [31–33]. Bacterial Co^{2+} and Ni^{2+} homeostasis systems have been studied with great detail in E. coli and to a lesser extent in other microorganisms [33]. As with copper, high levels of Co^{2+} and Ni^{2+} may exert toxic effects on microorganisms [23, 34]. The main mechanism of Co^{2+} and Ni^{2+} toxicity probably relates to their potential interference with iron (and possibly manganese) homeostasis. As

for most divalent metal cations, the main tolerance bacterial strategies to cope with excess Co^{2+} and Ni^{2+} are usually associated with membrane efflux systems.

Cobalt and nickel resistance systems have not been studied directly in pseudomonads. However, the identification of homologous genes for metal cation resistance in the genomes of species of Pseudomonas indicates that these bacteria have the potential to display tolerance mechanisms against Co^{2+} and Ni^{2+}. Co^{2+} resistance is usually accompanied by resistance either to Ni^{2+}, Cd^{2+}, or Zn^{2+}.

Two RND systems from megaplasmids of Cupriavidus metallidurans (previously named Alcaligenes eutrophus and Ralstonia metallidurans), the cnrCBA and nccCBA operons, confer resistance to both Co^{2+} and Ni^{2+} [4, 35, 36]. The CnrCBA system from plasmid pMOL28 is formed by the three typical RND polypeptides: CnrA, an inner membrane transporter, CnrC, located in the outer membrane, and CnrB, a membrane fusion protein bridging the periplasmic space [37]. This tripartite complex functions as a chemiosmotic pump driven by the proton-motive force that effluxes the cations probably from the cytoplasm to the periplasm and then to the outside (Fig. 9.1C). Additional cnrYXH genes regulate the expression of the efflux pump [38, 39]. The NccCBA complex is structurally and functionally similar to CnrCBA and is also regulated by corresponding nccYXH genes [40] (Fig. 9.1C). Unlike Cnr, the Ncc system, besides Co^{2+} and Ni^{2+} resistances, also confers resistance to Cd^{2+}.

The CzcCBA complex from plasmid pMOL30 of C. metallidurans, the first characterized RND system related to heavy metals, confers resistance to Cd^{2+}, Co^{2+} and Zn^{2+} [4, 41, 42] and will be described below in the cadmium section. A variant of this system, the Czn complex from Helicobacter pylori, has a distinct substrate specificity, exporting Cd^{2+}, Zn^{2+} and Ni^{2+} [43]. Also located in the Czc determinant is CzcD, a member of the CDF family, originally reported as a regulatory protein [44] but later found to confer low resistance to Co^{2+}, Cd^{2+} and Zn^{2+} [30]. Similar CDF transporters related to cation efflux, DmeF and FieF, have been identified in the C. metallidurans chromosome [45]. An interesting interplay between the Czc/Cnr RND systems and CDF proteins has been reported. CDFs seem to first export the cations from the cytoplasm to the periplasm and then RNDs pump them from the periplasm to the outside [45–47].

Transporters of the MFS group have been also assigned functions in Co^{2+} or Ni^{2+} efflux. This includes the first MFS protein found to be involved in metal transport, NreB of Achromobacter xylosoxidans, only transporting Ni^{2+} [48], and RcnA from E. coli which effluxes Co^{2+} and Ni^{2+} [49] (Fig. 9.1C). NreB and RcnA are histidine-rich polypeptides displaying a distinct topology of 12 and six transmembrane segments, respectively. The RcnR repressor regulates the expression of RcnA [50]. RcnA has been found to be also controlled by the global regulator Fur and was proposed to function as a connector of cobalt, nickel and iron homeostasis [51]. The P-type ATPase ZntA from E. coli, which confers cation resistance by the efflux of Zn^{2+}, Cd^{2+} and Pb^{2+} (see the sections of these metals below), is stimulated by Co^{2+} and Ni^{2+} [52] and may also efflux these ions although with little efficiency.

The genomes of P. aeruginosa [3] and P. putida [6] contain structural and regulatory czc genes (two copies in P. putida) which are probably involved in the efflux of

Co^{2+} and other divalent cations. Accumulation of Ni^{2+} as a resistance strategy has been reported in strains of P. aeruginosa [53] and P. putida [54], but the mechanisms involved have not yet been studied.

9.2.3 Zinc

A widely distributed enzyme cofactor, zinc displays afÐnity for ligands possessing oxygen, nitrogen or sulfur. As mentioned for copper, toxicity of zinc is associated with its ability to replace other metals (i.e. Ni^{2+}, Co^{2+}, Mn^{2+}) from enzymes or by forming complexes with other biomolecules. Zinc homeostasis has been studied with detail in several bacterial species [55]. Zinc occurs naturally as the divalent cation Zn^{2+} and the level of the metal is regulated by processes of Zn^{2+} uptake, sequestration by metallothioneins (MT), and efÑux from the cytoplasm [56].

The pumps of Zn^{2+} efÑux are usually not restricted to Zn^{2+} as a substrate, and may also catalyze transport of other divalent cations [56]. Zn^{2+} is exported across the cytoplasmic membrane by the P-type ATPase ZntA, described in E. coli [57], and by its closest homologue, CadA, Ðrst described in Staphylococcus aureus [58]. ZntA was the Ðrst example described of a speciÐc Zn^{2+} transporting protein in E. coli [57], but now is known to transport a broad range of soft metal ions, including Cd^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+} and Cu^{+} [59] (Fig. 9.1E). ZntA is a protein of 732 amino-acid residues with all the characteristics of a soft metal ion-translocating P-type ATPase, which include a cysteine-rich hydrophilic amino-terminal region that contains a single metal-binding motif $GMDC_{AA}C$ [56]. ZntA is regulated by ZntR (the zntR gene is located in another region of the chromosome in E. coli), that belongs to the MerR family of regulators. The expression of ZntA is induced by Zn^{2+}, Cd^{2+} and Pb^{2+}, being Cd^{2+} the more effective inducer [60, 61]. ZntR functions as a dimeric protein and tightly binds to its cognate promoter, P_{zntA}, located upstream of the zntA start codon [56]. A well-characterized system of Zn^{2+} transport is the CzcCBA complex of C. metallidurans [4], described below in the cadmium section.

Another protein that has been associated with zinc resistance is ZitB, a CDF transporter that mediates efÑux of Zn^{2+} in E. coli [62] (Fig. 9.1E). ZitB is closely related to CzcD that transports Cd^{2+}, Zn^{2+} and Co^{2+} [30] (described in the cadmium section). The expression of the zitB gene leads to a signiÐcant increase in Zn^{2+} resistance and to reduced Zn^{2+} accumulation in zntA-disrupted E. coli cells [62]. It has been proposed that ZitB contributes to Zn^{2+} homeostasis at low concentrations of zinc, while ZntA is required for growth at higher concentrations [62].

Zinc-regulated genes have been analyzed in Pseudomonas Ñuorescens employing mutagenesis [63]. One of the genes identiÐed was a zntA-like gene that was inducible by the presence of Zn^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, Hg^{2+}, and Ag^{+} ions. A mutant in this gene exhibited only hypersensitivity to Zn^{2+}, Cd^{2+} and Pb^{2+}, suggesting that it encodes a transporter for these cations. The P. putida strain S4 employs a dual strategy for zinc resistance [64]. One strategy is mediated by an inducible ATPase that efÑuxes the ion during the exponential phase of growth. The second mechanism is the accumulation of Zn^{2+} that can be stored by proteins in the outer membrane and the periplasm.

In addition to membrane transport pumps, some bacteria produce metallothioneins (MT) [15]. MTs are small poly-thiol proteins that bind metal cations, lowering their free concentrations within the cytoplasm (Fig. 9.1D). The best characterized prokaryotic MT is SmtA from Synechococcus PCC 7942, which protects against elevated levels of Zn^{2+} [65, 66]. For a long time SmtA was the only prokaryotic MT identified [67], but currently other related bacterial MTs, called BmtA, have been described [68]. MTs were purified from P. putida and P. aeruginosa strains and found that they were associated with three to four Zn^{2+} atoms [68]. Additional BmtA-like proteins were identified in P. fluorescens strains pf01 and SBW25. Most bacterial MTs identified to date have been found in cyanobacteria and pseudomonads [68].

9.3 Toxic Cations

This group of elements includes heavy metals with no known biological function and clear toxic effects over living cells: cadmium, lead and mercury. Potentially toxic metals which are irrelevant in biological terms, mainly by their presence at very low levels or in non available forms in most environments (i.e. gold, thallium, aluminum), will not be considered in this review. As with the essential cations, transmembrane efflux systems are also used by bacteria as key resistance mechanisms against toxic metal cations. Mercury represents a unique case for which intracellular sequestering followed by detoxification has evolved as a best suited bacterial tolerance strategy.

9.3.1 Cadmium and Lead

Cadmium chemistry is closely related to that of essential zinc. Cadmium and lead commonly form cations of oxidation states II, although lead may also exist in the IV valence. Lead differs from cadmium and zinc in their chemical coordination properties. In contrast to zinc, cadmium and lead bind preferentially sulfur (soft) ligands. Due to their similarity, zinc homeostasis and cadmium and lead resistance mechanisms often overlap, as reflected by their sharing of uptake and efflux transporters and metal-responsive regulatory proteins [59]. Intracellular Cd^{2+} is maintained at low levels through the control of sequestration or efflux of the ion. Cd^{2+} can be effluxed from bacterial cells by at least three systems: the P-type ATPase CadA, a large single polypeptide, the CzcCBA system, a three-polypeptide chemiosmotic RND complex that functions as an ion/proton exchanger, and CzcD, a single CDF membrane protein acting as a chemiosmotic efflux pump [15].

The P-type ATPase CadA from pI258 plasmid of S. aureus is the most studied Cd^{2+} resistance system [58]. CadA homologs have been found in several bacterial species, including pseudomonads. The system is localized in the cadAC operon. CadA catalyzes the active efflux of Cd^{2+}, Zn^{2+}, or Pb^{2+} [58, 69], and contains all the characteristic domains of a P-type ATPase [70, 71] (Fig. 9.2A). CadC is a transcriptional regulator needed for full Cd^{2+} and Zn^{2+} resistance in S. aureus [72].

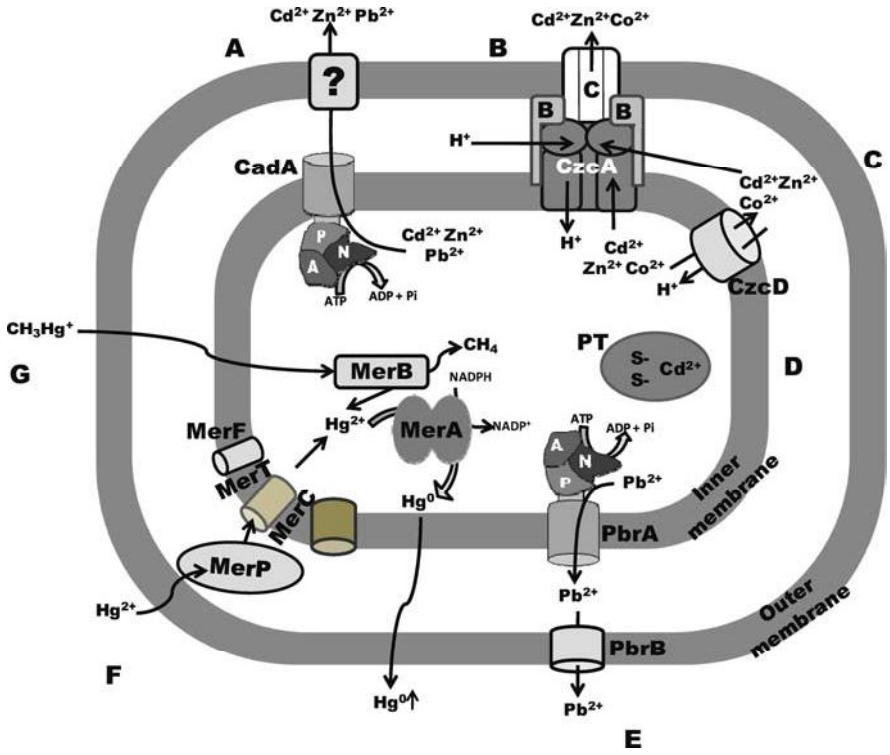

Fig. 9.2 Mechanisms of resistance to toxic cations in pseudomonads. A, cadmium P-type ATPase CadA. B, multication RND complex CzcCBA. C, CDF transporter CzcD. D, cadmium pseudothioneins (PT). E, Pbr lead resistance system. F, mercury resistance Mer system. G, organomercurial resistance MerB enzyme. P-type ATPases domains shown are: P, phosphorylation domain; N, nucleotide-binding domain; A, activator and phosphatase domain. The functions of the resistance systems are described in the text

The best studied homologue of CadA, the above mentioned ZntA from E. coli, is 30% identical to CadA.

The level of resistance to Cd^{2+} varies widely within species of Pseudomonas [73, 74]. P. putida 06909 possesses the cadA and cadR genes that are homologs to zntA and zntR from E. coli, respectively. CadA from P. putida 06909 confers a high level of resistance to Cd^{2+}, partial resistance to Zn^{2+}, and, unlike ZntA, does not confer Pb^{2+} resistance [75]. The level of Cd^{2+} resistance conferred by the cadA gene in P. putida 06909 is 17-fold higher than that conferred by zntA in E. coli. Homologous ORFs PA3690 (cadA) and PA3689 (cadR) are also present in the genome of P. aeruginosa PAO1, but their function has not been elucidated yet. CadA sequences from P. putida and P. aeruginosa both have a histidine-rich N-terminal extension that is missing in other CadA sequences; this region is probably responsible for the higher level of resistance to Cd^{2+} of these strains [75]. CadR is a transcriptional regulator of the MerR family [76]. CadR represses its own

expression in the absence of Cd^{2+}, it is induced in the presence of Cd^{2+}, and is necessary for full resistance to Cd^{2+} and Zn^{2+} [75]. P. putida KT2440 possesses the CadA1 and CadA2 transporters. CadA2 confers resistance to Cd^{2+} and Pb^{2+} in P. putida whereas CadA1 does not seem to confer metal tolerance in P. putida, but confers Zn^{2+} resistance when overexpressed in E. coli. CadA1 expression is inducible by Zn^{2+}. CadA2 is considered a housekeeping resistance mechanism against Cd^{2+} and Pb^{2+} [77]. CadA2 is constantly expressed at a high level even when Cd^{2+} is absent, but its expression increases in the presence of metals.

The second Cd^{2+} resistance determinant, the CzcCBA system, actively transports Cd^{2+}, Zn^{2+} and Co^{2+} out of the bacterial cell [15] (Fig. 9.2B). One of the best characterized systems is the czc determinant from C. metallidurans [36]. The system is organized like other three-component RND transporter complexes. CzcA is a cation/proton antiporter located in the cytoplasmic membrane that efÑuxes toxic cations to the periplasm [78]. CzcA is essential for cation transport and is considered the core of the complex. CzcB is a membrane fusion protein which spans the periplasmic space, bringing the outer and inner membranes in close position. The third component, CzcC, is an outer membrane protein that efÑuxes cations from the periplasm to the outside [59]. The CzcCBA system catalyzes the efÑux of both toxic and essentials cations and, for that reason, is tightly regulated by downstream and upstream regulatory regions.

Czc homologues have been detected by Southern hybridization in several Pseudomonas strains, including P. aeruginosa PAO1 [29]. The system is annotated as czcCBA-like in the PAO1 strain genome and probably confers resistance to Zn^{2+}, Cd^{2+}, and Co^{2+} [3]. In the environmental isolate P. aeruginosa CMG103, the czrSRCBA gene cluster confers a high level of resistance to Cd^{2+} and Zn^{2+} [29]. In P. aeruginosa PT5 (a PAO1 derivative) cross-resistance between heavy metal and antibiotic pumps has been reported [74]. The two-component system CzcS-CzcR controls the expression of the Czc efÑux pump and also regulates negatively the expression of the OprD porin, leading to carbapenem resistance. A czcCBA system is also functional in P. putida KT2440, which possesses two copies of the transporter [77]. CzcCBA1 confers Zn^{2+} resistance and its expression is induced by the metal; Cd^{2+}, and possibly Pb^{2+}, are also transported by CzcCBA1, but is less efÐcient when it acts as a Cd^{2+} or Pb^{2+} transporter. CzcCBA2 also confers Zn^{2+} resistance, but its expression is not induced by any metal.

The third Cd^{2+} resistance determinant, CzcD, is an efÑux pump that belongs to the CDF protein family [30] (Fig. 9.2C). The function of CzcD has been analyzed only in C. metallidurans but there are homologs in the genomes of P. aeruginosa PAO1 [3] and P. putida KT2440 [6]. CzcD is located in the cytoplasmic membrane and possesses at least six transmembrane helices. The level of resistance to Cd^{2+}, Zn^{2+} and Co^{2+} conferred by CzcD is lower as compared to that conferred by CzcA. CzcD is also involved in the regulation of the expression of the CzcCBA efÑux system.

A distinct cadmium resistance mechanism reported in Pseudomonas involves cadmium-binding proteins called pseudothioneins (PT) [79] (Fig. 9.2D). PTs have been identiÐed in a P. putida strain adapted to grow in 3 mM cadmium.

Pseudothioneins CdPT1, CdPT2 and CdPT3 are synthesized in different growth phases, being CdPT1 the major protein produced during the exponential phase. As metallothioneins, PTs are small cysteine-rich proteins (3.5Ã7 kDa). PTs have a lower cysteine content than mammalian metallothioneins (12Ã23% compared to 33%), but have in common that bind Cd^{2+}, Zn^{2+} and Cu^{2+} [79].

ZntA of E. coli and CadA of S. aureus also confer Pb^{2+} resistance in E. coli cells expressing the corresponding genes. A mutant strain with a disruption of the zntA gene showed hypersensitivity to Pb^{2+}; expression of cadA from pI258 plasmid complemented the phenotype, indicating that cadA also confers Pb^{2+} resistance [69].

The only speciÐc mechanism of Pb^{2+} resistance described so far is the pbr system, reported in C. metallidurans CH34 [80] (Fig. 9.2E). In contrast to the cad and znt systems, which only comprise the ATPase with a regulatory gene, the pbr system is constituted by several genes arranged in the divergent operons pbrUTR and pbrABCD. These operons encode proteins involved in three different processes: uptake, efÑux and accumulation of lead, which together confer maximal Pb^{2+} resistance. The role of PbrU on lead resistance has not been elucidated. PbrT is a permease that takes up Pb^{2+}; expression of pbrT alone in the absence of the pbrABCD genes results in Pb^{2+} hypersensitivity, due to an increase in Pb^{2+} uptake. The pbrR gene encodes the PbrR repressor that belongs to the ArsR/SmtB family of regulators. PbrR controls transcription of the pbr structural genes. PbrA is an inner membrane P-type ATPase, closely related to the CadA and ZntA ATPases, that efÑuxes Pb^{2+} to the periplasm. PbrB is an outer membrane lipoprotein that probably functions in removing Pb^{2+} from the periplasmic compartment, assisting PbrA for lead resistance [80, 81] (Fig. 9.2E). PbrC is probably an aspartic peptidase that removes the signal peptide from PbrB before it is transported to the periplasmic space; PbrC is required with PbrB for full resistance [80, 81]. PbrD is an intracellular protein that may bind Pb^{2+} with a cysteine-rich metal-binding motif but is not essential for Pb^{2+} resistance [80]. The Pbr system has not yet been identiÐed in Pseudomonas strains. In the P. putida KT2440 genome, of a total of 61 open reading frames with a putative role in metal homeostasis and detoxiÐcation, there seems not to be homologues to pbr genes [6].

9.3.2 Mercury

Mercuric ions (Hg^{2+}) display a rather strong affÐnity for sulfur-containing ligands, thus its toxicity relates mostly to protein sulfhydryl poisoning and to binding to other relevant thiol compounds. A main ubiquitous resistance mechanism is used by bacterial cells to tolerate mercury: the reduction of Hg^{2+} to Hg^{0}. This biotransformation converts highly toxic cationic mercury into the metallic species, an almost innocuous volatile form (Fig. 9.2F). Methylation of mercury will not be included in this chapter as does not seem to represent a resistance mechanism; this microbial modiÐcation often yields toxic organomercurial derivatives.

Mercury resistance has been reported since many years in Pseudomonas species [82, 83], including P. cepacia, P. Ñuorescens, P. putida, P. putrefaciens, and P. stutzeri [84]. The reduction of Hg^{2+} to Hg^0 is mediated by the mercuric reductase encoded by the merA gene, which is located in the mercury resistance mer operon [85, 86]. The mer operon is often encoded on mobile genetic elements [84]. The simplest and most studied Gram negative mer determinant is that from transposon Tn501, originally identiÞed in P. aeruginosa [82]. The mer operon shows several genetic arrangements depending on its origin. The mer operon of Tn501 consists of merRTPADE genes which encode polypeptides with regulatory, transport and enzymatic functions; other mer genes, such as merB, C, F, E and G, are localized on different mer operons [84, 86]. The mer genes are widely distributed among pseudomonads as shown in Fig. 9.3.

Bacteria	Locus	mer cluster
P. aeruginosa UCBPP-PA14	15460	
P. aeruginosa PA7	0104	
P. aeruginosa plasmid pVS1 , transposon Tn501	merA	
P. putida W619	2338	
P. stutzeri plasmid pPB	merA	
P. aeruginosa 07-406 plasmid pMATVIM-7	pMATVM7_04	
P. aeruginosa plasmid R1033	merA	
P. aeruginosa PA7	0090	
P. aeruginosa 2192	02098	
Pseudomonas sp. CT14 plasmid pCT14	pCT14_p48	
P. fluorescens plasmid pMER327	merA	
P. putida MU10-2	merA2	

Fig. 9.3 Schematic representation of the arrangement of the mer genes located in the genomes of pseudomonads. The columns indicate the Pseudomonas strain and the microbial locus for each merA gene. Boxed arrows indicate genes and the direction of transcription. Numbers below genes indicate the COG family to which each gene belongs [87]

The resistance mechanism consists of the initial binding of Hg^{2+} ions by a pair of cysteine residues on MerP, a mercury binding protein located in the periplasm of Gram-negative bacteria [88]. Mercuric ions are then transferred via a redox exchange mechanism to a pair of cysteines on MerT, an inner membrane transport protein (Fig. 9.2F). MerT is present in most mer operons from Gram negative bacteria. MerT is essential for mercury resistance and is the only Mer protein that interacts directly with MerP. Other Hg^{2+} membrane transporters are encoded by merC and merF genes. Deletion of merC and merF had no effect on the mercury resistance level [86]. Hg^{2+} is Þnally transferred via cysteine residues to the N-terminal domain of MerA, the homodimeric Ñavoprotein mercuric reductase,

the key component of the mercury detoxification system. The enzyme catalyzes the two-electron reduction of Hg^{2+} to volatile elemental mercury (Hg^0), which is nonenzymatically removed from the growth medium (Fig. 9.2F). The MerA enzyme utilizes NADPH as an electron donor and requires an excess of exogenous thiols for activity. MerA is located in the cytoplasm, where NADPH is abundant [85, 86].

In some bacteria, resistance to organomercurial compounds is also conferred by mer operons encoding the additional organomercurial lyase enzyme MerB (Fig. 9.2G). MerB is a monomeric cytosolic enzyme that cleaves the Hg–C covalent bond of both alkyl and aryl mercurials, releasing Hg^{2+}, which is then transformed by the MerA mercuric reductase. The activity of the MerB enzyme was first determined in Pseudomonas K-62 [86, 89]. Bacteria possessing MerB are tolerant to both inorganic and organic mercurials (broad-spectrum resistance); in contrast, the narrow-spectrum resistance determinants, where the mer systems lack merB, only confer resistance to Hg^{2+} [90]. Organomercurials are highly lipid-soluble as to enter the cell efficiently without a specific uptake system. The product of the merG gene lies in the periplasm and probably reduces permeability to organomercurials in soil pseudomonads [86]. Deletion of merG in Pseudomonas K-62 resulted in a decrease in phenylmercury resistance [91]. The regulatory genes for the mer system are merR and merD [86]. MerR belongs to the large MerR family that, as mentioned above, includes transcriptional regulators for Cd^{2+}, Zn^{2+}, Cu^{2+} and Pb^{2+}. MerR is an activator of the mer cluster and in Gram negative bacteria is divergently transcribed from the major mer promoter [76].

9.4 Toxic Oxyanions

Arsenic and chromium are toxic nonessential metalloids that may be present as environmental pollutants. The main tolerance mechanism developed by bacteria for arsenic and chromium oxyanions is their efflux from the cytoplasm by specific orthologous membrane transporters. Oxyanions derived from essential elements that generally lack toxicity (i.e. molybden and tungsten) or those that, while showing toxicity commonly occur at very low levels in the environment (uranium and vanadium) are not described here.

9.4.1 Arsenic

Arsenic is a metalloid present in numerous disturbed and natural ecosystems. It can exist in multiple oxidation states, with the most common being arsenite [As(III)] and arsenate [As(V)]. Although some microorganisms can utilize As(V) for anaerobic respiration [92] or oxidize As(III) as a sole energy source, arsenic is generally toxic to most microbes [93]. Arsenate (AsO_4^{3-}) is a toxic analog of phosphate (PO_4^{3-}), and most organisms take up arsenate via phosphate transporters [94]. As(V) toxicity is due to the uncoupling of ATP phosphorylation, that would directly impact energy flow, as well as to the inhibition of nucleic acid and phospholipid syntheses

[95]. In bacteria, the pathway for uptake of trivalent metalloids as As(III) is through the polyol transporter GlpF, which belongs to the family of aquaglyceroporins [96]. As(III) toxicity is predominantly due to its ability to covalently bind protein sulfhydryl groups [93]. Arsenate and arsenite (AsO_2^-) oxyanions are detoxified by an interplay of redox, transport, sequestration, and covalent modification reactions [94] (Fig. 9.4A).

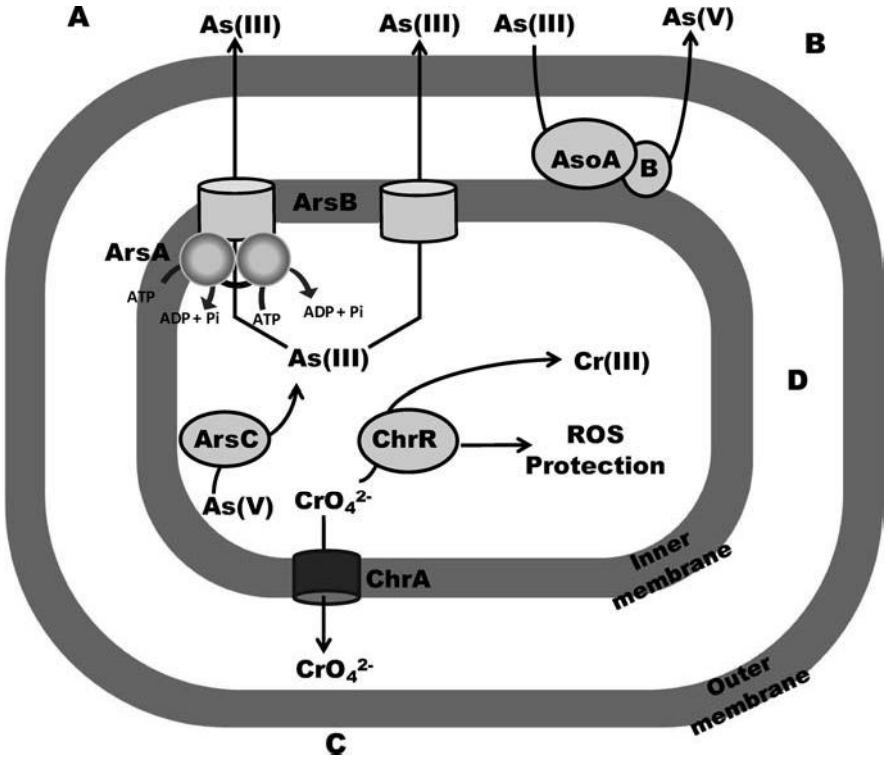

Fig. 9.4 Mechanisms of resistance to toxic oxyanions in pseudomonads. A, arsenic resistance ArsABC and ArsBC systems. B, AsoAB arsenite oxidase complex. C, chromate resistance ChrA transporter. D, ChrR chromate reductase. The functions of the resistance systems are described in the text

Bacteria adapt to arsenic toxicity mainly by the development of resistance mechanisms conferred by chromosomal or plasmid-encoded arsenical resistance (ars) operons [96]. Once the trivalent form of the metalloid accumulates in the cell, resistance is produced by their removal from the cytosol [96]. The ars clusters are widely distributed among pseudomonads, as shown in Fig. 9.5.

The mechanism of resistance to arsenic conferred by ars genes has been best characterized from E. coli plasmid R733 [97]. The ars operon consists of genes arsRDABC. The arsA gene encodes the ATPase enzyme subunit of a protein complex composed of an ArsA dimer bound to ArsB, an inner membrane polypeptide

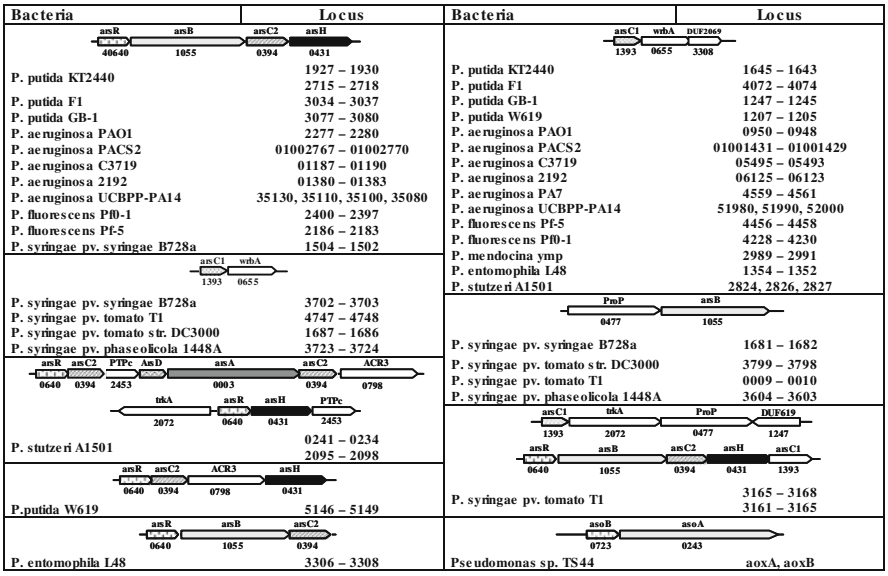

Fig. 9.5 Schematic representation of the local genomic context of ars and aso genes located in the genomes of pseudomonads. All ars and aso genes shown are located in chromosomes. The columns indicate the Pseudomonas strain and the microbial locus for each gene. Preliminary information on aso genes is also included. Boxed arrows indicate genes and direction of transcription. Numbers below genes indicate the COG family to which each gene belongs. [87]

(Fig. 9.4A). As(III) is the substrate of the ArsAB efÑux pump, which is an As(III)-translocating ATPase [97]. ArsB alone is sufÐcient for As(III) resistance and proton motive force-dependent As(III) efÑux; bacteria lacking ArsA are still resistant to arsenic [96] (Fig. 9.4A). arsC1 belongs to the COG1393 family [87] and encodes an enzyme that reduces As(V) to As(III), which is subsequently extruded from the cell; arsenate reductase activity is required for optimal resistance to As(V) [93] (Fig. 9.4A). In vitro, the reductase activity requires both reduced glutathione (GSH) and any of the three E. coli glutaredoxins, Grx1, Grx2 or Grx3 [96]. The arsD gene is constitutively expressed and encodes a regulatory protein that controls the maximal expression of the ars operon [93]. Finally, arsR encodes a repressor that controls the expression of the ars operon and can be induced by As(III), Sb(III) or even bismuth.

ars homologous sequences have been identiÐed in chromosomal DNA from P. aeruginosa [98]. Phylogenetic analysis showed that P. aeruginosa and P. putida possess arsC chromosomal homologs [99]; in the case of P. aeruginosa, its genome contains separate genes for glutaredoxin- and thioredoxin-coupled ArsC reductases [96]. The chromosome of P. Ñuorescens MSP3 possesses a less complex arsenic operon (arsRBC) which confers resistance to arsenate and arsenite [100].

Analysis of the P. putida KT2440 genome revealed two very similar systems, arsRBCH, for arsenic resistance [6]. The distinct gene arsH is located downstream of arsC and is transcribed in the same direction. The arsH gene product of Yersinia

enterocolitica was reported to confer resistance to both arsenite and arsenate and was assigned a possible role as a transcriptional regulator [101]. However, P. putida arsH genes, denominated ArsH1 and ArsH2, showed a signiÞcant similarity to plant NADH oxidoreductases and to Bacillus subtilis azoreductase [6]. ArsH is widely distributed in bacteria and sparsely in fungi, plants, and archaea [96], but its role in arsenic resistance is still unclear.

Bacterial oxidation of As(III) to less toxic As(V) may be considered as a resistance mechanism. Arsenite oxidase (Aso) from Alcaligenes faecalis is the best understood example of this detoxiÞcation activity [94]. The enzyme is formed by a molybdopterin-containing subunit and a Fe-S Rieske subunit encoded by the asoA and asoB genes, respectively [102] (Fig. 9.4B). The aso genes form part of the so called Åarsenic gene islandÆ encoding proteins related to arsenic resistance and homeostasis; these include putative periplasmic oxyanion binding proteins, probably associated with ABC membrane transporters, as well as an arsenite efÑux pump (ArsAB) ATPase. An ortholog of the AsoA Mo-pterin subunit of arsenite oxidase was identiÞed in the genome of Pseudomonas sp. TS44 (Fig. 9.5), suggesting that some pseudomonads also possess the ability to oxidize arsenite [102].

As(III) toxicity via a mechanism involving peroxidation of unsaturated fatty acids was found in P. putida [103, 104]. It was proposed that this process leads to the generation of organic hydroperoxides and oxygen radicals, which induce components of the oxidative stress response such as superoxide dismutase (SOD) and catalase [93]. Catalase activity increased in response to the presence and oxidation of As(III) [104]. These studies also showed that the levels of glutathione reductase (Gor) increased upon exposure of P. putida to As(III). One function of Gor in E. coli is to recycle oxidized glutathione back to reduced glutathione, which is the reductant for the As(V) reductase that converts As(V) to As(III). The latter is then actively removed from the cell by the ArsB effÑux pump [93].

Mutants of P. aeruginosa PAO1 affected in the arsB, crc (the catabolite repressor control protein) and gor genes are more sensitive to As(III) than wild-type strain [93]. The crc mutant was more sensitive to H_2O_2 in the presence of As(III); the sensitivity to As(III) was assumed to be due to an abnormal regulation of genes under Crc control. Double sodA/sodB mutants also exhibited increased sensitivity to As(III), suggesting that the oxidative stress response is involved in As(III) resistance [93].

9.4.2 Chromium

The biological effects of chromium depend on its oxidation state. At the extracellular level, Cr(VI) (usually in the form of chromate, CrO_4^{2-}) is highly toxic to most bacteria, whereas Cr(III) is relatively innocuous by its inability to traverse cell membranes. In the cytoplasm, chromium toxicity is mainly related to the process of reduction of Cr(VI) to lower oxidation states [i.e. Cr(III) and Cr(V)] in which free radicals may form [105, 106]. Bacterial resistance determinants may be encoded either by chromosomal genes or by plasmids [107, 108]. Usually genes located

on plasmids encode membrane transporters which directly mediate efÑux of chromate ions from the cytoplasm. On the other hand, resistance systems encoded within bacterial chromosomes are generally related to speciÐc or unspeciÐc Cr(VI) reduction, free-radical detoxifying activities, repairing of DNA damage, and processes associated with sulfur or iron homeostasis [106].

The best understood mechanism of resistance to chromate is the efÑux of $C_rO_4^{2-}$ conferred by the ChrA protein encoded by the P. aeruginosa plasmid pUM505 [109]. ChrA is a membrane protein of 416 amino acid residues which displays a topology of 13 transmembrane segments (TMS) [110]. ChrA functions as a chemiosmotic pump that efÑuxes CrO_4^{2-} from the cytoplasm using the proton motive force [111, 112] (Fig. 9.4C). Plasmid pMOL28 from C. metallidurans [113], plasmid 1 from Shewanella sp. ANA-3 [114], and transposon TnOtChr from Ochrobactrum tritici 5bv11 [115] all encode ChrA homologs which confer resistance to chromate. The resistance mechanism seems to be the same for these homologs from proteobacteria, as all caused reduced accumulation of CrO_4^{2-}.

Structure-function analyses have been conducted with the P. aeruginosa ChrA protein. Random mutagenesis of the chrA gene showed that most essential amino acids are located in the amino terminal end of ChrA [116]. In agreement with this Ðnding, phylogenetic analysis of ChrA homologs revealed that the amino terminal halves are more conserved than the carboxyl halves [117], suggesting that the two halves of ChrA carry out different functions in the transport of CrO_4^{2-}.

The ChrA proteins belong to the CHR superfamily of transporters, Ðrst described by Nies et al. [107] as a small group of prokaryotic proteins involved in CrO_4^{2-} and sulfate (SO_4^{2-}) transport. This superfamiliy was classiÐed as TC no. 2.A.51 [118] and includes proteins encoded in chromosomes and plasmids. The protein databases currently contain several dozens of homologs, including proteins from eukaryotes, and has been named as the CHR superfamily of chromate ion transporters [117]. The CHR superfamily contains two families, large proteins (comprising seven LCHR subfamilies) and short proteins (comprising three SCHR subfamilies). All pseudomonads with genomes sequenced have ChrA homologs from subfamilies LCHR1 or LCHR5, as well as other chr related genes (Fig. 9.6).

Plasmids pB4 [119], from a Pseudomonas sp. strain, and pUM505, from P. aeruginosa [106], possess chrBAC gene clusters that share high sequence similarity with the resistance determinant from plasmid pMOL28. A function of the ChrB protein in the inducibility of the chrA gene by CrO_4^{2-} had been previously demonstrated in C. metallidurans [113]. The presence of chrC, a gene encoding a probable SOD enzyme, is another variable feature of the chr gene clusters [120] (Fig. 9.6).

A second mechanism of resistance to CrO_4^{2-} is the transformation of hexavalent chromium to the trivalent form [105, 108]. Microbial reduction of Cr(VI) to Cr(III) is not a plasmid-associated trait. Chromate reduction has been demonstrated in diverse pseudomonad species, including P. ambigua [121], P. Ñuorescens [122] and P. putida [123], although only a few enzymes have been characterized to date [105]. Initial studies suggest that chromate reductases may have a different primary role other than chromate reduction; this secondary function for chromate reductases

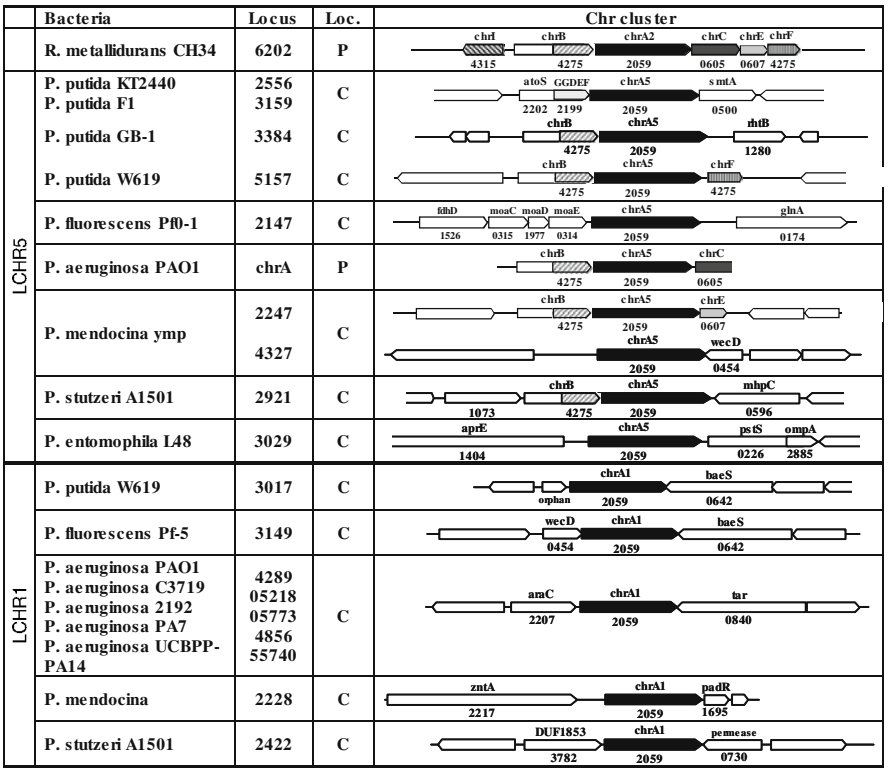

Fig. 9.6 Schematic representation of the local genomic context of chr genes located in the genomes of pseudomonads. All chrA genes belong to the LCHR1 or LCHR5 subfamilies of the CHR superfamily [117]. The columns indicate the Pseudomonas strain, the microbial loci and the location (Loc.) for each chrA gene (P, plasmid; C, chromosome). Boxed arrows indicate genes and direction of transcription. IdentiÐed genes are indicated according to the characterized chr determinant from C. metallidurans plasmid pMOL28. Numbers below genes indicate the COG family to which each gene belongs [87]

may be related to the recent introduction of Cr(VI) to the environment by industrial pollution.

The currently best studied chromate reductase is the ChrR enzyme from P. putida, a soluble Ñavin mononucleotide-binding enzyme which reduces Cr(VI) to Cr(III) [124] (Fig. 9.4D). PuriÐed ChrR revealed that a quinone reductase activity produced a Ñavin semiquinone during CrO_4^{2-} reduction; this activity transferred >25% of the NADH electrons to reactive oxygen species (ROS) and generated the Cr(V) species transiently. This property of ChrR provides an antioxidant defense mechanism to P. putida by shielding cells against H_2O_2 toxicity [125] (Fig. 9.4D). ChrR in one pathway reduces Cr(VI) to Cr(III) generating intermediary Cr(V) and ROS, and, by an additional mechanism, reduces quinones to protect against ROS. ChrR from P. putida belongs to the NADP(H)-dependent FMN reductase (FMN_red) protein family, currently comprising about 250 homologs [106].

Besides chromate efÑux and reduction, several other resistance mechanisms to deal with chromium are displayed by bacteria. Since oxidative stress is responsible for most toxic efects of chromate, protection and detoxiÐcation systems against this process is an important part of the defensive barrier. Protection of bacterial cells from DNA damage caused by CrO_4^{2-} is another defensive shield. Cr(VI) has long been known to induce the E. coli SOS repair system that protects DNA from oxidative damage [126]. DNA helicases RecG and RuvB, and the FtsK protein, components of DNA repair and chromosome segregation processes, have ben shown to participate in the response to DNA damage caused by CrO_4^{2-} exposure in P. aeruginosa [127, 128]. Additional protective strategies may be related with sulfur or iron metabolism, but these systems have not been analyzed in pseudomonads.

9.4.3 Tellurium

Tellurium is a rare-earth metalloid with a low abundance in the EarthÈs crust but which derivatives may be pollutants in industrial waste discharges. The tellurite oxyanion (TeO_3^{2-}) is highly toxic for most microorganisms, particularly Gram-negative bacteria [129]. Tellurite toxicity in E. coli is several orders of magnitude higher than that of heavy metals such as cobalt, zinc and chromium [23, 130]. Studies on TeO_3^{2-} metabolism and toxicity in E. coli showed that the oxyanion interacts with reduced thiols and that glutathione is the initial target of tellurite reactivity [131]. Tellurite, as chromate, is reduced intracellularly producing toxic intermediates which may cause DNA damage [132].

Despite several genetic determinants related to TeO_3^{2-} resistance have been analyzed to the molecular detail, a general mechanism to explain this phenotype is not available [15, 133]. Instead, a variety of possible biochemical strategies used by bacteria to defend themselves from tellurite toxicity have been reported.

Antibiotic-resistant clinical isolates of enterobacteria and P. aeruginosa commonly possess plasmids conferring TeO_3^{2-} resistance [134, 135]. A TeO_3^{2-} resistance determinant from the chromosome of P. syringae pv. pisi encodes a methyl transferase enzyme that may detoxify tellurium by methylation [136]. Similarly, the P. putida genome contains genes that may encode tellurium (and selenium) methylation activities [6]. P. aeruginosa PAO1 mutants affected in the genes encoding DNA helicases RuvB, RecG and the DNA translocase FtsK, that function in DNA repair and chromosome segregation, respectively, showed an increased susceptibility to tellurite [127, 128]. Protection from DNA damage caused by TeO_3^{2-} exposure was proposed as the role of those enzymatic activities in conferring TeO_3^{2-} resistance. The precipitation of TeO_3^{2-} by a siderophore, pyridine-2, 6-bis(thiocarboxylic acid), produced by P. stutzeri KC has been proposed as another mechanism for TeO_3^{2-} detoxiÐcation [137]. A detailed metabolomics study of the tellurite hyperresistant Pseudomonas pseudoalcaligenes KF707 strain revealed that the resistance phenotype involves a variety of complex cell modiÐcations, including the induction of the oxidative stress response, resistance to membrane alterations, and a rearrangement of cellular metabolism [138].

The tehAB operon from the E. coli chromosome encodes TehA, an integral membrane protein, and TehB, a weakly membrane-associated protein [139]. A possible role for this operon in the efÑux of TeO_3^{2-} has been discarded [140]. In contrast, the E. coli ArsAB ATPase, which efÑuxes arsenite, is also able to transport TeO_3^{2-} [141]. The TehAB system confers TeO_3^{2-} resistance by a distinct strategy involving thiol redox enzymes, such as glutathione reductase and thioredoxin reductase, as a mechanism of oxidative protection [139]. The TehB protein was found to bind S-adenosyl methionine as a methylation cofactor that detoxiÐes TeO_3^{2-}, as mentioned above for the P. syringae tellurium resistance determinant [142]. Other E. coli tellurite resistance systems, the kilA and ter determinants, seem to function by protecting glutathione from being reduced by TeO_3^{2-} [131].

9.5 Other Toxic Ions

To close the listing of heavy metals displaying deleterious effects on bacteria, a brief outline will be given next for the environmentally important elements silver, tin and selenium, for which resistance mechanisms have been only barely analyzed in pseudomonads.

Silver is a highly toxic metal with several biomedical uses, mainly as an antiseptic. Even though numerous examples of silver resistance have been reported in pseudomonads [143Ã145], no studies on resistance mechanisms have yet appeared. As mentioned in the section of copper, the genomes of P. aeruginosa [3] and P. putida [6] possess homologs for the CusCBA system, a proton-driven RND transporter which may efÑux Ag^+ from the cytoplasm [24], and for SilP, a P-type ATPase which extrudes Ag^+ (see below) [25]. A rather complex Ag^+ resistance system, Ðrst described from a Salmonella plasmid [25, 146], was later found widely spread in other enterobacterial plasmids [147]. It consists of three operons. silE encodes the Ag^+-inducible periplasmic SilE protein, which binds Ag^+ ions and prevents their entry to the cytoplasm. Next, the silCFBAP operon encodes the P-type ATPase SilP, which transports Ag^+ from the cytoplasm to the periplasm, the RND complex SilCBA, an efÑux pump able to extrude periplasmic Ag^+ to the outside, and SilF, a periplasmic chaperone that escorts Ag^+ from SilP to the SilCBA pump. A third operon, silRS, encodes a typical two-component regulatory system that controls the expression of the Sil system.

Tin is a nonessential metal whose inorganic forms have little toxicity. In contrast, organotins, widely used organometallic compounds, are highly toxic for microorganisms [148]. Organotin-resistant bacteria have been isolated from polluted ecosystems [148, 149]. Several pseudomonad strains resist organotins by breaking Sn-C bonds [150, 151]. A distinct resistance mechanism is displayed by a P. stutzeri strain possessing the tbtABM operon [152]. TbtABM is a RND system which efÑuxes tributyltin from the cytoplasm. TbtABM also confers resistance to antibiotics and aromatic compounds and shows homology with P. putida multidrug resistance systems. No further details on this organotin resistance mechanism have been reported.

Selenium, a metalloid related to sulfur and tellurium, is required as a micronutrient by most microorganisms. However, toxic selenium oxyanions selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) may be generated by industrial activities. The reduction of selenite (and less frequently of selenate) to elemental Se^0, considered as a detoxification mechanism, may be carried out by varied bacterial species [153–155], including pseudomonads [156]. Heavy metal-resistant C. metallidurans has the ability to reduce selenite to Se^0, which accumulates as granules in the cytoplasm [155]. The P. putida genome contains genes that may encode selenium methylation activities [6]. No genes or biochemical mechanisms are available for bacterial selenium resistance.

9.6 Concluding Remarks

Pseudomonads have evolved diverse resistance mechanisms to cope with heavy metal toxicity. Due to the distinct chemical properties of each toxic metal(loid), and to the different levels to which the microorganisms are exposed, bacteria with varied defense systems have been selected. Strategies involving the exclusion of cytoplasmic ions by membrane efflux pumps seem to be a preferred mechanism. Except for mercury, and probably for tellurite, all the toxic ions treated here may be the substrates of efflux pumps. These transporters belong to a variety of membrane protein families (RND, CDF, MFS, P-type ATPases) frequently widespread among all life domains. A second common resistance mechanism involves the use of redox enzymes. For most chemical elements susceptible to generate different valence species with a lower toxicity (i.e. mercury, arsenic, chromium, selenium), redox detoxification systems are usually deployed by pseudomonads. As may be inferred from the resistance systems described above, understanding the interactions of pseudomonads with toxic heavy metals largely benefited from biochemical and genomic information generated in other bacteria. A conclusion that may be drawn is that the relatively large genomes of pseudomonads are plenty of genetic determinants for heavy metal resistance, which is in tune with the diverse environments that these bacteria use to inhabit.

Acknowledgments Research in authors' laboratories was funded by Coordinación de Investigación Científica (UMSNH), Consejo Nacional de Ciencia y Tecnología (No. 79190), COECYT (Michoacán), and DGAPA-UNAM (IN:208308).

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