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Metal Response in *Cupriavidus metallidurans*

Volume I: From Habitats to Genes and Proteins

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Preface

This two-volume set is a collaborative work aimed to review the *Cupriavidus metallidurans* resistance mechanisms to toxic concentrations of heavy metals at the ecological, physiological, genomic, transcriptomic and proteomic level. The main heavy metals studied are zinc, nickel, cadmium, cobalt, copper, chromium (chromate), lead, mercury, gold and silver. *C. metallidurans*, a soil β-proteobacterium belonging to the *Burkholderiaceae*, is very well adapted to high concentrations of heavy metals and is able to survive in a variety of harsh oligotrophic habitats linked to industrial and other human activities. This volume contains three chapters, each with its own emphasis. Chapter 1 discusses anthropogenic waste as a source of metal-resistant *Cupriavidus* together with mobile genetic elements as vectors of metal-responsive genes and possible actors in evolution driven by the adaptation to such environments. Chapter 2 reviews the genomic context of the metal response genes in *C. metallidurans* CH34 with a focus on its mobile genetic elements. Chapter 3 inventories the catalogues of metal resistance genes, proteins and mechanisms as well as some environmental applications. Mechanisms first discovered in this bacterium such as the RND efflux pumps for cadmium, cobalt, nickel and zinc, and the cation diffusion factors (with CzcD being one of the first identified) are highlighted together with the resistance determinants to other metals such as chromate, lead, mercury, silver and gold, as well as the intricate regulatory network and accessory genes. Some of these accessory genes are exclusively found in *C. metallidurans* and are likely involved in the adaptation to very high heavy metal concentrations. Volume II reviews the available structural data of *C. metallidurans* proteins involved in heavy metal resistance.

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scientists, who participated in the *Cupriavidus* (*Alcaligenes*, *Ralstonia*,…) work started with Prof. Jean Remacle at the University of Liège and further carried out in Mol [Belgian Centre for Nuclear Energy (SCK•CEN) and Flemish Institute for Technological Research (VITO)] during four decennia, and to the directorates of the corresponding institutions for their interest and support.

Mol, Belgium

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Chapter 1
The History of *Cupriavidus metallidurans* Strains Isolated from Anthropogenic Environments

Max Mergeay

Abstract The multiple metal-resistant *Cupriavidus metallidurans* strain CH34 is quite a latecomer on the scene of transposon- and plasmid-mediated resistance to heavy metals. Yet, straightaway it was remarkable because of its combined resistance to cadmium, cobalt, zinc, copper, nickel and lead, and the first genetic determinants for tripartite chemiosmotic cation/proton efflux systems and cation diffusion factors. Later, other resistances and interesting satellite genes attracted the attention and the presence of orthologs for most of the metal resistance genes already described in other bacteria became apparent. A long time designated as *Alcaligenes eutrophus*, this β-proteobacterium belongs to the *Burkholderiaceae* and got its definitive name after diverse taxonomic vicissitudes. The first isolates of this species were obtained from Belgian industrial sites in the Meuse basin for which the historical and geographical context is briefly sketched to illustrate the interactions between bacteria and human activities, and the possible evolutionary consequences on bacterial genomes especially as far as the association of metal resistance genes with mobile genetic elements is concerned. Other *C. metallidurans* isolates were found in a variety of industrial sites around the world up to China and Australia and up to the International Space station, and even in clinical isolates. Besides the *C. metallidurans* CH34 genome, other genomes of *C. metallidurans* and *Ralstonia pickettii* strains containing metal resistance genes are available. The *C. metallidurans* CH34 genome displays a peculiar richness in mobile genetic elements (genomic islands, transposons and insertion sequences spread on the four replicons: the chromosome, the chromid (formerly second chromosome) and the megaplasmids). Genetic and ecological studies on mobile genetic elements and the metal resistance genes carried by them are also significant in the general context of horizontal gene transfer and the dispersion of epidemiological important genetic determinants as those conferring antibiotic resistance or pathogenicity.

Keywords *Cupriavidus metallidurans* · Isolation · Biotopes · Anthropogenic environments · Mobile genetic elements

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1.1 The Shaping of *Cupriavidus metallidurans*

In 1976, *Cupriavidus metallidurans* type strain CH34 was isolated from sediments in a decantation basin in the non-ferrous metallurgical plant at Engis, located near Liège (Belgium) in the Meuse valley (Mergeay et al. 1978) (Fig. 1.1), and became a

Fig. 1.1 Map showing the River Meuse basin, including the network of canals linking the Belgian Meuse with the Port of Antwerp. (*1*) The "Vieille Montagne/Altenberg" mine, where zinc resistance plasmids were captured in a plasmid-free *C. metallidurans* strain (The Plombières site, 1998; Fig. 1.2); (*2*) Liège, The "Vieille Montagne" factory at Angleur, cradle of the modern zinc industry; (*3*) Engis, The "Métallurgie de Prayon" factory, where *C. metallidurans* CH34 was isolated (1976); (*4*) The old Benedictine Abbey at Flône; (*5*) Dinant, the centre of medieval brass metallurgy and archaeological site of brass smelting ovens; (*6*) Verdun, an archaeological site of brass metallurgy, isolation site of *Cupriavidus* sp. B9 (2013); (*7*) Overpelt, non-ferrous metallurgy, isolation site of *C. basilensis* ER121 (1990); (*8*) Lommel, "Usine de Campine", isolation site of *C. campinensis* DS185 (1990); (*9*) Beerse, non-ferrous metallurgy, isolation site of *C. metallidurans* SV661 (1990)

model organism to study heavy metal resistance and tolerance (Mergeay et al. 1985; Mergeay et al. 2003; Monchy et al. 2007; Janssen et al. 2010; Nies and Silver 1995; Nies 1999, 2003; von Rozycki and Nies 2009). Initially, strain CH34 was assigned to the genus *Pseudomonas*, however, at that time the taxonomy of this genus was already undergoing a full and radical reassessment (Palleroni 2003; Stanier et al. 1966). Subsequently, strain CH34 was classified as a close relative of the facultative chemolithotroph *Alcaligenes eutrophus* (Schlegel et al. 1961), as both strains express soluble and particulate hydrogenases (Lejeune et al. 1983; Mergeay et al. 1985; Mergeay et al. 1978). Later, the taxonomic proximity of CH34 to plant pathogens such as *Ralstonia solanacearum* (Yabuuchi et al. 1995) was also recognized as well as to some strains that are able to efficiently degrade xenobiotic organics (Don and Pemberton 1981; Springael et al. 1993; Springael et al. 1994; Don and Pemberton 1985).

Other strains isolated from a variety of industrial biotopes were able to survive high heavy metal concentrations (Brim et al. 1999; Diels and Mergeay 1990; Goris et al. 2001; Mergeay 2000; Schmidt and Schlegel 1994; Schmidt et al. 1991; Stoppel et al. 1995; Stoppel and Schlegel 1995; Timotius and Schlegel 1987) and shared typical genetic features with strain CH34, especially plasmid-borne resistances to nickel (Sensfuss and Schlegel 1988; Liesegang et al. 1993; Grass et al. 2001; Schmidt and Schlegel 1994) cobalt (Liesegang et al. 1993; Schmidt and Schlegel 1994), lead (Borremans et al. 2001), chromate (Juhnke et al. 2002; Peitzsch et al. 1998; Nies et al. 1998), mercury (Diels et al. 1985), cadmium, cobalt, and zinc (Borremans et al. 2001; Diels et al. 1985; Juhnke et al. 2002; Liesegang et al. 1993; Monchy et al. 2007; Nies 1995; Nies et al. 1987; Nies et al. 1989; Peitzsch et al. 1998; Ryan et al. 2009; Sensfuss and Schlegel 1988), which led to further and rather drastic taxonomic re-evaluations and additions. In the case of strain CH34, the taxonomic classification changed from *Alcaligenes* via *Ralstonia* and *Wautersia* into the genus *Cupriavidus* (Makkar and Casida 1987) and seems to be stabilized now (Sato et al. 2006; Vandamme and Coenye 2004; Vaneechoutte et al. 2004; Chen et al. 2001). However, older or different genus or species names are still used, for instance in the recent papers on the catabolic strain JMP134 or the reference chemolithoautotrophic strain H16 (Schwartz and Friedrich 2001; Pohlmann et al. 2006; Fricke et al. 2009).

Finally, to summarize, the *Cupriavidus* genus (class β-Proteobacteria, family *Burkholderiaceae*) is described as a group of Gram-negative, aerobic rods that are motile by means of peritrichous flagella, form smooth colonies that reach 1–2 mm within 48 h at 30 °C on blood agar, are susceptible to colistin, and neither acidify nor assimilate glucose. The *C. metallidurans* species is described as a group of short rods (0.8 × 1.2–2.2 μm) that are oxidase- and catalase-positive, do not produce indole from tryptophan, assimilate D-gluconate, adipate and L-malate but not D-glucose, L-arabinose, D-fructose, D-mannose, D-mannitol, N-acetyl-D-glucosamine or maltose. For full descriptions of both the genus and species see Vandamme and Coenye (2004) and Vaneechoutte et al. (2004).

1.2 Strain CH34 in Historical and Geographical Context

C. metallidurans and closely related strains were typically isolated from industrial biotopes contaminated with heavy metals, such as the decantation basin in the non-ferrous metallurgical plant at Engis where strain CH34 was isolated (Mergeay et al. 1978). The plant at Engis was located in the province of Liège (Belgium) on the river Meuse and at a short distance from the former Abbey of Flône (founded in 1074), which profited from neighbouring mining, including that of zinc ores (calamine, see below). From the middle ages up to the 18th century, these ores were used for the production of brass and in the copper- and brassware trade for which the Meuse valley and especially Dinant were renowned. Brass, an alloy of copper and zinc, was developed before production methods for metallic zinc were known. Calamine brass was the first type of brass produced, probably starting during the 1st millennium BC, and was not replaced in Europe by other brass manufacturing until the 18th century (it is likely that Indian brass manufacturers, Benares was renowned for brassware, had developed more advanced techniques some centuries earlier). At the beginning of the 12th century, brass metallurgy, including accurate technical descriptions of brass metalworking processes, was described in a composite treatise entitled "Diversarum artium schedula" written under the pseudonym Theophilus likely corresponding to one or more Benedictine monks living in Germany, with metal production centres at that time being Goslar, north of the Rammelsberg mine on the northern edge of the Harz mountains, and Erzgebirge (Krušné hory; the Ore Mountains) in Bohemia (Gearhart 2010; Clarke and Stijnman 2012).

Brass was produced by heating a mixture of copper and calamine to a high temperature for several hours allowing zinc vapour to distil from the ores and permeate the metallic copper. This process is known as cementation. Since the late 10th century, brass (also with some addition of tin and lead) was used for the production of pots and pans for local usage and export, and also to make gold work for religious purposes by major artists of the Mosan region such as Renier de Huy and Nicolas de Verdun (12th century) or Renier van Thienen (15th century). Recently, archaeological studies of medieval metallurgy have shed light on the brass metallurgy in the Meuse valley, especially in Dinant, the centre of medieval brass metallurgy exporting "dinanderie" in Northern and Western Europe, but also in Verdun where sediments highly contaminated with zinc and copper dating from the 12th to 16th century were found at a depth of 2 m in the remnants of metallurgical furnaces and workshops. Archaeological campaigns in urban sites (so called preventive archaeology) have extensively studied such sites and even hypothesized signs of microbial intervention (Müller et al. 2011). Preliminary microbiological analysis of the Verdun sediments revealed the presence of a *Cupriavidus* strain resistant to zinc, cadmium and copper (Gillan, Thomas, Vremard, Provoost, Wattiez and Mergeay, in prep.).

Later, this region surrounding Liège and along the Meuse became a major centre during the Industrial Revolution in the early 19th century (because of the local development of zinc, and later non-ferrous and ferrous, metallurgy) when calamine (actually a mixture of the virtually indistinguishable zinc ores smithsonite ($ZnCO_3$) and hemimorphite ($Zn_4Si_2O_7(OH)_2 \cdot H_2O$)) was processed using the method discovered by Jean-Jacques Dony (1759–1819) in 1810. The mineral calamine takes its name from La Calamine/Kelmis in Belgium (with the site of Plombières; Fig. 1.2) where the "Altenberg/Vieille Montagne" mine was located and where it has been mined since the medieval period. This region with his strategic mining resources gained geopolitical value and became a source of conflict as it was on the border of Prussia and the Netherlands (15 years later divided into the Kingdoms of Netherlands and Belgium). In 1815, the area containing the mine was designated by the Congress of Vienna, busy to reshape Europe after the French Revolution and Napoleonic wars, as a minuscule neutral territory (Moresnet) at the border between Belgium, Germany and the Netherlands, which issued its own stamps and became a kind of Esperanto language laboratory before its incorporation into Belgium in 1919 after the First World War and the subsequent treaty of Versailles.

Although it is not self-evident, the history and archaeology of metallurgy may be fully relevant from the view point of soil microbiology, as metallurgical processes and diverse metalworking applications produce waste with variable levels of bioavailable metals and create localized environments that can be exploited by metal-adapted microorganisms in various kinds of soils.

Throughout this book, anthropogenic waste could be the main thread tying together products or artefacts of historical, artistic, cultural or economic value and soil microbiology. Indeed, metallic waste is a long-lasting waste without equal, as unlike organic wastes, metals are immutable. In addition, weathering of some ores (serpentinization), natural leaching, and volcanic and geothermal release of heavy metals (Boyd and Barkay 2012) also create conditions for the selection and natural

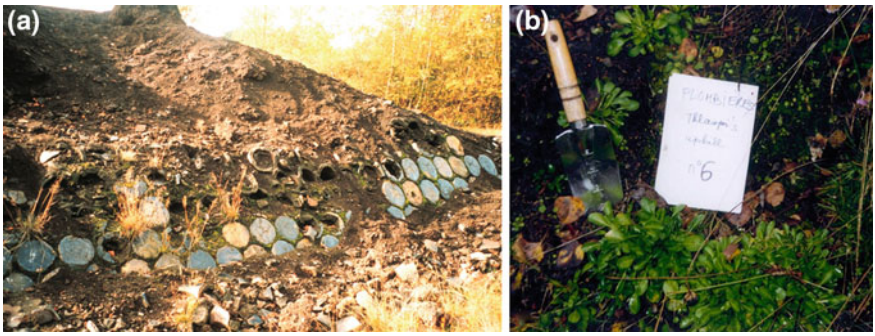

Fig. 1.2 Natural reservation of a site on a lead, zinc and silver foundry exploited in Plombières (Bleiberg), Belgium up to 1922. **a** Wall of foundry residues; **b** Sampling of a soil covered by metallophytes (*Noccaea* (previously *Thlaspi*) *caerulescens* subsp. *calaminare*). Metal content in mg/kg: Zn, 18450; Pb, 4450; Cu, 211; Ni, 77

enrichment of metal-resistant bacteria that could have contributed to the ancestral patrimony of modern strains found in anthropized environments before the massive importation of ores from other continents.

As both brass and zinc metallurgy in the Meuse valley relied on the same zinc ores, it is likely that selection of metal-resistant bacteria occurred earlier than the Industrial Revolution or the zinc metallurgy based on Dony's manufacturing process and the posterior explosive development of non-ferrous metallurgy. It must be added that, in the 20th century, the Belgian non-ferrous metallurgy mainly relied on ores from other regions, especially from Katanga (Congo), since local ores were almost exhausted.

1.3 Other *C. metallidurans* Strains Closely Related to Strain CH34

1.3.1 Germany, Japan and Central Africa

Strains very similar to *C. metallidurans* CH34 have also been found in soils around other metallurgical plants in North-Eastern Belgium and the Congo (Katanga) (Diels and Mergeay 1990; Van Houdt et al. 2012) as well as from contaminated soils in Japan (Kunito et al. 1996) and sewage plants in Germany (Timotius and Schlegel 1987; Schmidt and Schlegel 1989; Schmidt et al. 1991).

As far as Katanga is concerned, most of the *Cupriavidus* strains known to this day come from wastes produced by the extensive metallurgical processing developed during the 20th century. However, the history of metallurgy in Central Africa dates back at least to the 4th century and is mainly known because of the production of cross-shaped copper ingots ("handa" or "croisettes") that were first used as a sign of power or dignity (as shown by their presence in ancient graves) and later as currency exported across the African continent and overseas (Bisson 2000). In the 19th century, just before the huge development of modern copper metallurgy in the colonial period of the 20th century, production was estimated to be around 40 tons per year, which should have produced waste and favourable conditions for the local enrichment of metal-adapted microbial populations. Copper metallurgists from Katanga (who designated themselves as "copper eaters") (de Hemptinne 1926; Herbert 1984) were organized in secret societies with a specific organization and rituals. Quite a lot of archaeological sites of the pre-colonial period (up to the late 19th century) containing remnants of furnaces and ovens have been identified and may be of interest for further microbiological studies (Bisson 2000; de Plaen et al. 1982), although some of these sites were recently destructed due to the proliferation of small mining operations (Guy de Plaen, pers. comm.).

1.3.2 New Biotopes in the 21st Century

1.3.2.1 From Greece to Australia and China

In the 21st century, additional biotopes for *C. metallidurans* or related metal-resistant bacteria have been described. In Australia, *C. metallidurans* was found to be one of the key organisms in biofilms on gold grains from three gold mining sites in Queensland including the Prophet gold mine in Kilkivan. This led to the discovery of biogenic gold and other observations on the response of *C. metallidurans* CH34 to gold (Fairbrother et al. 2013; Reith et al. 2006, 2007, 2009).

Metal-resistant *Cupriavidus* sp. BIS7 and *Cupriavidus* sp. HMR-1 were isolated from a tropical soil in Malaysia and a heavy metal-enriched culture of activated sludge from a wastewater treatment plant in Hong Kong, respectively. *C. metallidurans* strain PD11 was isolated from waste water from a chemical laboratory at Okayama University (Japan) and was shown to degrade dichloromethane (Miyake-Nakayama et al. 2004; Miyake-Nakayama et al. 2006).

C. metallidurans strain XXKD-1 was isolated from the wall of a 475 m deep subsurface laneway in the Qixiashan lead-zinc sulphide mine in Eastern China (near to Nanjing in the Jiangsu Province of China) (Zhao et al. 2012). Strain XXKD-1, which was collected from the microbial biofilms found on the tufa, was shown to bioaccumulate heavy metals (especially zinc, cadmium and manganese). Bioprecipitation (of calcium carbonate) was also shown for *C. metallidurans* strain B_MPEZ isolated from marble stone from an ancient marble quarry in Penteli Mountain (Athens, Greece) (Daskalakis et al. 2013; Daskalakis et al. 2014). These observations could match those of bioprecipitation and metal removal from culture supernatants that were reported for strain CH34 (Pumpel et al. 2003; Diels et al. 1993, 2003, 2009). Both the XXKD-1 and B_MPEZ strains may contribute to the genomic comparison (with some focus on the mobile genetic elements) with those strains that were found in the wastes from chemical and metallurgical factories from the Industrial Revolution or in other strongly anthropized environments.

Using culture-independent molecular techniques many sequences related to the 16S rRNA gene of strain CH34 or to its genes involved in metal resistance have been retrieved from different environments. For instance, similar 16S rRNA gene sequences were retrieved from Cr(VI) and Cr(III)-contaminated industrial wastewaters (Hellenic Aerospace Industry, Viotia, Greece) (Katsaveli et al. 2012), uranium-contaminated subsurface sediments (Oak Ridge National Laboratory, Tennessee, USA) (Akob et al. 2007), borehole water and a granite rock core from a >1000-m-deep molybdenum mine (Henderson Mine, Colorado, USA) (Sahl et al. 2008), and unsterilized razor blades (Sohrabi et al. 2011). Sequences 99 % identical to the *nreB* gene of plasmid pMOL30 were among other found in a nickel-enriched batch reactor grown with seed sludge from a Shanghai sewage treatment plant (Li et al. 2011).

1.3.2.2 Spacecraft-Related Environments: Clean Rooms and the International Space Station

C. metallidurans and related *Ralstonia pickettii* strains containing the *czc* genes (resistance to cadmium, zinc and copper) identical to those of plasmid pMOL30 were also unexpectedly found during microbiological analysis of the clean rooms where spacecraft are built and that are subjected to rigorous biocontamination eradication, monitoring and control programs (Mijnendonckx et al. 2013). *C. metallidurans* NE12 was recovered from an air filter from the Kennedy Space Center payload hazardous servicing facility (Florida) (Newcombe et al. 2008) and *R. pickettii* SSH1, SSH2, SSH3 and SSH4 from the surface of the Mars Odyssey orbiter (Jet Propulsion Laboratory Spacecraft assembly and encapsulation facility (Pasadena, California) (La Duc et al. 2003). Furthermore, *C. metallidurans* NA1, NA2 and NA4, and *R. pickettii* CW1, CW2, CW3 and CW4 were isolated from the International space Station (ISS) drinking (Bobe et al. 2008) and cooling water (Benardini et al. 2005), respectively. The genomes of *C. metallidurans* strains NA1, NA4 and NE12 (Monsieurs et al. 2014), and *R. pickettii* strains SSH4 and CW2 were sequenced and are available for comparative analysis. The discovery of these strains also emphasizes the characteristics of these genera that are not directly linked to metal resistance but rather to survival and recovery from oligotrophic conditions.

1.4 *Cupriavidus* and *Ralstonia* as Opportunistic Pathogens

Interestingly, *C. metallidurans* isolates are increasingly being recovered from clinical sources such as cystic fibrosis patients (Coenye et al. 2005), human cerebrospinal fluid (e.g. strain CCUG43015, deposited directly to the Culture Collection of the University of Göteborg) and the pharmaceutical industry (e.g. strain CCUG45957, direct deposition). It remains to be studied if these strains were causing an active infection or intervened only as secondary opportunistic pathogens (Coenye et al. 2005). However, recently, a first case of invasive human infection was reported as *C. metallidurans* was isolated from four out of five blood culture samples taken from a patient suffering a significant nosocomial septicaemia (Langevin et al. 2011; Monsieurs et al. 2013). In addition, other *Cupriavidus* species, including *Cupriavidus taiwanensis* and *Cupriavidus basilensis*, have been recovered from clinical sources such as cystic fibrosis patients (Chen et al. 2001; Coenye et al. 2005). *Cupriavidus pauculus* and *Cupriavidus gilardii* have been described as a cause of human infections (Stovall et al. 2010; Karafin et al. 2010; Christensen et al. 2010) as well as the closely related *R. pickettii* species (Ryan et al. 2006).

1.5 Other *Cupriavidus* Species Resistant to Heavy Metals: *C. campinensis* and *C. basilensis*

Strains from other *Cupriavidus* species such as *C. campinensis* and *C. basilensis* carrying genes similar to *czc* from pMOL30, which confers resistance to cadmium, cobalt and zinc, have been isolated from various non-ferrous metallurgical sites in North-Eastern Belgium (Fig. 1.1) For instance, *C. campinensis* DS185 was isolated from a "zinc desert" in Lommel (Belgium) and *C. basilensis* ER121 in the metallurgical plant of Overpelt (Brim et al. 1999; Goris et al. 2001; Diels and Mergeay 1990; Diels et al. 1989; Diels et al. 1995). Their genome sequences are not yet available, however, at least one *C. basilensis* genome has recently been sequenced (Cserháti et al. 2012). Plasmid pMOL85 of *C. campinensis* DS185 and plasmid pMOL65 of *C. basilensis* ER121 were transferred at high frequency to plasmid-free derivatives of *C. metallidurans* CH34 by selecting for metal resistance. Restriction and DNA hybridization analysis of plasmids pMOL85 and pMOL65 showed that the corresponding *czc* genes are highly similar to those of pMOL30 (Diels et al. 1989; Diels et al. 1995). In addition, *C. campinensis* DS185 carries a second large plasmid conferring resistance to copper, which was exploited to construct a luminescence-based biosensor (Corbisier et al. 1996; Corbisier 1997; Corbisier et al. 1999; Diels et al. 1994).

1.6 Bacterial Resistance to Heavy Metals and Mobile Genetic Elements

The *C. metallidurans* genome (based on type strain CH34) is especially rich in genes involved in the resistance and adaptation to high heavy metal concentrations as found in metallurgical waste from industrial environments (especially zinc, copper, cadmium, lead and nickel) (Mergeay et al. 2003; Janssen et al. 2010). The corresponding genes are mostly associated with mobile genetic elements (MGEs) such as plasmids, transposons, genomic islands and other actors in horizontal gene transfer (Monchy et al. 2007; Van Houdt et al. 2009; Van Houdt et al. 2012), all of them being gathered under the mobilome concept (Frost et al. 2005). The mobilome allows its host to settle in specific niches or to adapt to harsh conditions thereby surviving rather extreme environmental conditions.

Plasmid-borne resistance to heavy metals has been described or suggested to be present in bacteria since the last third of the 20th century, especially in isolates from clinical and agricultural environments. The first reports described small or medium size plasmids (transposons) from *Staphylococcus aureus* and *Escherichia coli* (Novick and Roth 1968) carrying genes for resistance to mercury, cadmium, lead, bismuth or arsenic (Hedges and Baumberg 1973; Mobley et al. 1983; Nakahara et al. 1977; Ni'Bhriain et al. 1983; Silver and Misra 1984; Smith and Novick 1972; Summers and Silver 1972, 1978; Novick and Roth 1968; Peyru et al. 1969; Silver

and Misra 1988; Silver 1996; Silver and Phung 1996). A copper-resistant plasmid, pRJ1004, was also found in an *E. coli* strain isolated from the manure of pigs fed with copper-supplemented feed (Tetaz and Luke 1983). Orthologs of the corresponding *pco* genes (designated *cop*), which are involved in periplasmic detoxification, were described in *Pseudomonas syringae* strains found on copper-treated vegetables and fruit in California (Cooksey 1993). Afterwards, many corresponding orthologs found in other bacteria (including *Cupriavidus*) were named according to the *P. syringae* nomenclature.

This period of intense research interest in bacterial resistance to toxic metals provided an important leap in our knowledge of the resistance mechanisms to cadmium, arsenic, copper and mercury. Mercury resistance is very frequently associated with transposons (Foster 1987; Misra 1992; Liebert et al. 1999) and some of the mercury resistance genes such as the regulatory *merR* gene or *merA* encoding the mercuric reductase remain the best known in the field and have become paradigms. Other mechanisms identified during the period before publication of metal-resistant β-proteobacteria extended from the seminal work of Novick and Roth (1968) in the late sixties to the middle eighties and included efflux P-type ATPases and the periplasmic copper detoxification mentioned above.

1.6.1 Mobile Genetic Elements and Human Activities

Before these observations on metal resistance genes, MGEs with determinants ensuring resistance to therapeutic antibiotics have been abundantly described since the 1950s and their massive dispersion in sensitive organisms may be considered a direct consequence of human activities (production in pharmaceutical industries and application to clinical environments) that created heavy pressures on microorganisms and subsequent evolutionary constraints. Julian Davies said at the end of the 20th century: "*No discussion of the past 50 years of antibiotic history can overlook the mortal combat between terrestrial microbes and the antimicrobial agents that humans have released into the environment. The past half-century provides a capsule of microbial evolution, the survival of the oldest living organisms in the face of yet another catastrophic situation*" (Davies 1999). Nevertheless, it should not be forgotten that the therapeutic, cosmetic and other uses (including deliberate poisoning) of mercury, arsenic, lead and other metals have been reported since the Antiquity (Egypt, China, Greece and Rome) up to the modern ages, which may be reflected in the linkage of metal resistance with MGEs in bacteria associated with human/animal ecosystems and pathologies.

Another emerging type of genes carried by MGEs are catabolic genes involved in the degradation of organic xenobiotics such as chlorinated aromatic and aliphatic industrial compounds. The appearance of these genes can reasonably be attributed to rather recent evolutionary changes in response to the appearance of manmade chemical compounds that had never existed before (Nojiri et al. 2014). These compounds produced by the chemical industry such as polychlorinated biphenyls,

monomers for the plastics industry, agricultural pesticides and other derivatives were initially considered as fully recalcitrant to biological degradation and were massively released in the environment. However, they became carbon sources for microbial consortia at first and finally for discrete bacterial species (Nojiri et al. 2014). The emergence and infective dispersion of catabolism of man-made xenobiotic organics in bacterial species clearly occurred since the last part of the 19th century and throughout the 20th century. Therefore, these genes became a major focus to study putative evolutionary processes in bacterial genomes in a time lapse compatible with our current experimental approaches. In this respect, the genus *Cupriavidus* contains the strain JMP134 (formerly *Ralstonia eutropha* JMP134) discovered in Australia (Don and Pemberton 1985) and a reference strain for the study of catabolic pathways as it hosts the broad host range (BHR) plasmid pJP4 encoding genes responsible for the degradation of 2,4-diphenoxyacetic acid (2,4-D), a herbicide and a typical xenobiotic chemical (Lykidis et al. 2010; Trefault et al. 2004; Matus et al. 2003; Perez-Pantoja et al. 2008; Perez-Pantoja et al. 2009). Recently, it was found that strain JMP134 is closely related to hydrogenotrophic *Cupriavidus* strains discovered in the dispersed volcanic ashes from the major eruption of Mount Pinatubo in the Philippines in July 1991 (Sato et al. 2006). These Mount Pinatubo strains were assigned to two novel *Cupriavidus* species: *C. pinatubonensis* (including JMP134) and *C. laharis* (lahar is a Javanese word that describes volcanic mudflows or debris flows) (Sato et al. 2006).

Another story of plasmid-mediated degradation of a herbicide produced by the chemical industry is that of atrazine (2-chloro-4-(ethylamine)-6-(isopropylamine)-s-triazine) by enzymes encoded by the IncP-1β plasmid pADP-1. The latter was first found in *Pseudomonas* sp. strain ADP (Martinez et al. 2001). The corresponding atrazine degradation genes were later also found in a *Ralstonia* strain (thus closely related to the *Cupriavidus* genus) (de Souza et al. 1998) and in actinobacterial *Arthrobacter* strains (Rousseaux et al. 2002). These genes (as well as those for degradation of other xenobiotics) are often flanked by IS*1071*-like insertion sequences (Sota et al. 2006). Together with BHR plasmids (such as the IncP-1α plasmids), IS*1071*-like elements mediate the widespread distribution of these genes in soil bacteria (Dunon et al. 2013; Kim et al. 2013). Degradation of atrazine and its use as a carbon and nitrogen source was also reported in a *C. basilensis* strain (Stamper et al. 2002). Degradation of xenobiotic compounds such as dichloromethane by *C. metallidurans* has been demonstrated for strain PD11 isolated in Japan (Miyake-Nakayama et al. 2006).

Besides the chemical and pharmaceutical industry, we also have to consider metallurgy, the key actor in the Industrial Revolution starting in Europe at the end of the 18th century. During the past three centuries, metallurgical processes produced huge amounts of waste that profoundly affected soil surfaces and freshwater sediments as well as their inhabitants, and likely to a much greater extent than since the great oxidation event, when the Earth's atmosphere became oxygenated and metals became soluble as a consequence of their oxidation. However, empiric metallurgy has also a long history encompassing some millennia before the Industrial Revolution. Every development in metallurgical processes (especially

with non-ferrous metals such as copper, tin, lead, gold, silver and likely also mercury, and later iron) had major economic, cultural and political consequences and produced long-term bioavailable waste of some toxicity (likely in amounts not comparable to those produced during the Industrial Revolution, but nonetheless in locally high concentrations). This is of consequence for the microbial soil populations and likely for their specific history at the genomic level (acquisition, amplification or rearrangements of toxic metal processing functions and corresponding genes as well as further dispersion in neighbouring populations). In this respect, much more attention should be given to the identification, inventory, effective protection (Bodelier 2011) and sound scientific exploitation (bioremediation, metagenomics, mobile genetic elements, metal speciation and fate) of metallurgical sites that have an important microbial component and are representative of anthropocene biotopes or anthromes (Martin et al. 2014). Such sites are especially fragile and predisposed to radical cleaning neither considering their microbial component nor their possible evolution to more complex associations with plants (metallophytes) and animals.

Of course, accessory genes carried by bacterial MGEs are not limited to genetic determinants influenced by rather recent human activities with nitrogen fixation, hydrogenotrophy and specific catabolisms being evident examples among many others. Furthermore, we cannot neglect those MGEs involved in microbial virulence against human and animal hosts. Such elements are intricately associated with events of human history and are infectiously dispersed through various and sometimes explosive kinds of human mobility such as wars, migrations, invasions, pilgrimages and commercial routes. Microbial virulence determinants very likely encompass the whole history of humanity, its (domestic) animals and their associated microflora, and may reflect a longer struggle with hosts that predate humans, much more than the genetic determinants influenced by human activities we have highlighted up to now (degradation of xenobiotics or resistance to industrially produced antibiotics or to heavy metals). They are also rooted in the whole evolutionary history of relationships between Bacteria, Archaea and Eukarya. Therefore, if MGEs harbouring antibiotic resistance, xenocatabolism or resistance to heavy metals emerged or re-emerged at different times in human history or became more apparent as a response to the last centuries' industrial and healthcare revolutions, we should not forget that most of the known MGEs were found in Proteobacteria, Firmicutes and Actinobacteria. These phyla may share the same environments and horizontal gene transfer of resistance or catabolic determinants to strains of public health concern carrying virulence determinants may occur despite their occupation of rather specific taxonomic and ecological territories. This would deserve extreme attention as genetic determinants for metal resistance (zinc and cadmium but also copper) were recently reported to regularly accompany multiple antibiotic resistance in methicillin-resistant *S. aureus* of porcine and human origin (Cavaco et al. 2010; Cavaco et al. 2011; Moodley et al. 2011; Gomez-Sanz et al. 2013). Therefore, the co-localization of antimicrobial and heavy metal resistance genes may facilitate their persistence, co-selection and dissemination (Gomez-Sanz et al. 2013).

References

Akob DM, Mills HJ, Kostka JE (2007) Metabolically active microbial communities in uranium-contaminated subsurface sediments. FEMS Microbiol Ecol 59(1):95–107

Benardini J, Ballinger J, Crawford R, Roman M, Sumner R, Venkateswaran A (2005) International space station internal active thermal control system: an initial assessment of the microbial communities within fluid from ground support and flight hardware. SAE Technical Paper: 01-3094

Bisson MS (2000) Precolonial copper metallurgy socio-political context. In: Bisson MS, Childs ST, de Barros P, Holl AFC (eds) Ancient Africa metallurgy: the socio-cultural context. Altamira press, Walnut Creek, pp 83–132

Bobe LS, Kochetkov AA, Soloukhin VA, Andreichuk PO, Protasov NN, Sinyak Y (2008) SRV-K status aboard the international space station during missions 15 and 16. SAE Technical Paper: 01-2191

Bodelier PL (2011) Toward understanding, managing, and protecting microbial ecosystems. Front Microbiol 2:80

Borremans B, Hobman JL, Provoost A, Brown NL, Van der Lelie D (2001) Cloning and functional analysis of the *pbr* lead resistance determinant of *Ralstonia metallidurans* CH34. J Bacteriol 183(19):5651–5658

Boyd ES, Barkay T (2012) The mercury resistance operon: from an origin in a geothermal environment to an efficient detoxification machine. Front Microbiol 3:349

Brim H, Heyndrickx M, de Vos P, Wilmotte A, Springael D, Schlegel HG, Mergeay M (1999) Amplified rDNA restriction analysis and further genotypic characterisation of metal-resistant soil bacteria and related facultative hydrogenotrophs. Syst Appl Microbiol 22(2):258–268

Cavaco LM, Hasman H, Aarestrup FM (2011) Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. Vet Microbiol 150(3–4):344–348

Cavaco LM, Hasman H, Stegger M, Andersen PS, Skov R, Fluit AC, Ito T, Aarestrup FM (2010) Cloning and occurrence of *czrC*, a gene conferring cadmium and zinc resistance in methicillin-resistant *Staphylococcus aureus* CC398 isolates. Antimicrob Agents Chemother 54(9):3605–3608

Chen WM, Laevens S, Lee TM, Coenye T, De Vos P, Mergeay M, Vandamme P (2001) *Ralstonia taiwanensis* sp nov., isolated from root nodules of mimosa species and sputum of a cystic fibrosis patient. Int J Syst Evol Microbiol 51:1729–1735

Christensen JB, Vitko NP, Voskuil MI, Castillo-Mancilla JR (2010) Implantable cardiac defibrillator pocket infection due to a previously undescribed *Cupriavidus* species. J Clin Microbiol 48(7):2623–2625

Clarke M, Stijnman A (2012) Around Theophilus: an expert meeting towards new standards in Theophilus scholarship. In: The artist's process: technology and interpretation: proceedings of the fourth symposium of the Art technological source research working group, London. Archetype, pp 215–217

Coenye T, Spilker T, Reik R, Vandamme P, Lipuma JJ (2005) Use of PCR analyses to define the distribution of *Ralstonia* species recovered from patients with cystic fibrosis. J Clin Microbiol 43(7):3463–3466

Cooksey DA (1993) Copper uptake and resistance in bacteria. Mol Microbiol 7(1):1–5

Corbisier P (1997) Bacterial metal-*lux* biosensors for a rapid determination of the heavy metal bioavailability and toxicity in solid samples. Res Microbiol 148(6):534–536

Corbisier P, Thiry E, Diels L (1996) Bacterial biosensors for the toxicity assessment of solid wastes. Environ Toxicol Water Qual 11(3):171–177

Corbisier P, van der Lelie D, Borremans B, Provoost A, de Lorenzo V, Brown NL, Lloyd JR, Hobman JL, Csoregi E, Johansson G, Mattiasson B (1999) Whole cell-and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples. Anal Chim Acta 387(3):235–244

Cserháti M, Kriszt B, Szoboszlay S, Tóth Á, Szabó I, Táncsics A, Nagy I, Horváth B, Nagy I, Kukolya J (2012) *De Novo* genome project of *Cupriavidus basilensis* OR16. J Bacteriol 194 (8):2109–2110

Daskalakis MI, Magoulas A, Kotoulas G, Catsikis I, Bakolas A, Karageorgis AP, Mavridou A, Doulia D, Rigas F (2013) *Pseudomonas*, *Pantoea* and *Cupriavidus* isolates induce calcium carbonate precipitation for biorestoration of ornamental stone. J Appl Microbiol 115(2):409–423

Daskalakis MI, Magoulas A, Kotoulas G, Katsikis I, Bakolas A, Karageorgis AP, Mavridou A, Doulia D, Rigas F (2014) *Cupriavidus metallidurans* biomineralization ability and its application as a bioconsolidation enhancer for ornamental marble stone. Appl Microbiol Biotechnol 98(15): 6871–6883

Davies J (1999) In praise of antibiotics. ASM News, vol 65

de Hemptinne J-F, Mgr (1926) Les Mangeurs de cuivre du Katanga. Revue générale de la Colonie belge 1(3):371–403

de Plaen G, Malaisse F, Brooks RR (1982) The copper flowers of Central Africa and their significance for prospecting and archeology. Endeavour 6(2):72–77

de Souza ML, Seffernick J, Martinez B, Sadowsky MJ, Wackett LP (1998) The atrazine catabolism genes *atzABC* are widespread and highly conserved. J Bacteriol 180(7):1951–1954

Diels L, Corbisier P, Hooyberghs L, Glombitza F, Hummel A, Tsezos M, Pumpel T, Pernfuss B, Schinner F, Mergeay M (1994) Heavy metal resistance and biosorption in *Alcaligenes eutrophus* ER121. In: Paper presented at the international biohydrometallurgy symposium

Diels L, Dong QH, Vanderlelie D, Baeyens W, Mergeay M (1995) The *czc* operon of *Alcaligenes eutrophus* CH34: from resistance mechanism to the removal of heavy metals. J Ind Microbiol 14(2):142–153

Diels L, Faelen M, Mergeay M, Nies D (1985) Mercury transposons from plasmids governing multiple resistance to heavy metals in *Alcaligenes eutrophus* CH34. Arch Physiol Biochem 93:27–28

Diels L, Mergeay M (1990) DNA probe-mediated detection of resistant bacteria from soils highly polluted by heavy metals. Appl Environ Microbiol 56(5):1485–1491

Diels L, Sadouk A, Mergeay M (1989) Large plasmids governing multiple resistances to heavy metals—a genetic approach. Toxicol Environ Chem 23(1–4):79–89

Diels L, Spaans PH, Van Roy S, Hooyberghs L, Ryngaert A, Wouters H, Walter E, Winters J, Macaskie L, Finlay J, Pernfuss B, Woebking H, Pumpel T, Tsezos M (2003) Heavy metals removal by sand filters inoculated with metal sorbing and precipitating bacteria. Hydrometallurgy 71(1–2):235–241

Diels L, Van Roy S, Taghavi S, Doyen W, Leysen R, Mergeay M (1993) The use of *Alcaligenes eutrophus* immobilized in a tubular membrane reactor for heavy-metal recuperation. In: Torma AE, Apel ML, Brierley CL (eds) International biohydrometallurgy symposium (IBS-93). Jackson Hole, Wy, 22–25 Aug 1993, pp 133–144

Diels L, Van Roy S, Taghavi S, Van Houdt R (2009) From industrial sites to environmental applications with *Cupriavidus metallidurans*. Anton Leeuw Int J G 96(2):247–258

Don RH, Pemberton JM (1981) Properties of six pesticide degradation plasmids isolated from *Alcaligenes paradoxus* and *Alcaligenes eutrophus*. J Bacteriol 145(2):681–686

Don RH, Pemberton JM (1985) Genetic and physical map of the 2,4-dichlorophenoxyacetic acid-degradative plasmid pJP4. J Bacteriol 161(1):466–468

Dunon V, Sniegowski K, Bers K, Lavigne R, Smalla K, Springael D (2013) High prevalence of IncP-1 plasmids and IS1071 insertion sequences in on-farm biopurification systems and other pesticide-polluted environments. FEMS Microbiol Ecol 86(3): 415–431

Fairbrother L, Etschmann B, Brugger J, Shapter J, Southam G, Reith F (2013) Biomineralization of gold in biofilms of *Cupriavidus metallidurans*. Environ Sci Technol 47(6):2628–2635

Foster TJ (1987) The genetics and biochemistry of mercury resistance. Crit Rev Microbiol 15(2):117–140

Fricke WF, Kusian B, Bowien B (2009) The genome organization of *Ralstonia eutropha* strain H16 and related species of the *Burkholderiaceae*. J Microbiol Biotechnol 16(1–2):124–135

Frost LS, Leplae R, Summers AO, Toussaint A (2005) Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 3(9):722–732

Gearhart HC (2010) Theophilus' *On Diverse Arts*: the persona of the artist and the production of art in the twelfth century. University of Michigan, Michigan

Gomez-Sanz E, Kadlec K, Fessler AT, Zarazaga M, Torres C, Schwarz S (2013) Novel *erm(T)*-carrying multiresistance plasmids from porcine and human isolates of methicillin-resistant *Staphylococcus aureus* ST398 that also harbor cadmium and copper resistance determinants. Antimicrob Agents Chemother 57(7):3275–3282

Goris J, De Vos P, Coenye T, Hoste B, Janssens D, Brim H, Diels L, Mergeay M, Kersters K, Vandamme P (2001) Classification of metal-resistant bacteria from industrial biotopes as *Ralstonia campinensis* sp nov., *Ralstonia metallidurans* sp nov and *Ralstonia basilensis* Steinle et al. 1998 emend. Int J Syst Evol Microbiol 51:1773–1782

Grass G, Fan B, Rosen BP, Lemke K, Schlegel HG, Rensing C (2001) NreB from *Achromobacter xylosoxidans* 31A Is a nickel-induced transporter conferring nickel resistance. J Bacteriol 183(9):2803–2807

Hedges RW, Baumberg S (1973) Resistance to arsenic compounds conferred by a plasmid transmissible between strains of *Escherichia coli*. J Bacteriol 115(1):459–460

Herbert EW (1984) Red gold of Africa: copper in precolonial history and culture. The University of Wisconsin Press, Madison

Janssen PJ, Van Houdt R, Moors H, Monsieurs P, Morin N, Michaux A, Benotmane MA, Leys N, Vallaeys T, Lapidus A, Monchy S, Medigue C, Taghavi S, McCorkle S, Dunn J, van der Lelie D, Mergeay M (2010) The complete genome sequence of *Cupriavidus metallidurans* strain CH34, a master survivalist in harsh and anthropogenic environments. PLoS ONE 5(5):e10433

Juhnke S, Peitzsch N, Hubener N, Grosse C, Nies DH (2002) New genes involved in chromate resistance in *Ralstonia metallidurans* strain CH34. Arch Microbiol 179(1):15–25

Karafin M, Romagnoli M, Fink DL, Howard T, Rau R, Milstone AM, Carroll KC (2010) Fatal infection caused by *Cupriavidus gilardii* in a child with aplastic anemia. J Clin Microbiol 48(3):1005–1007

Katsaveli K, Vayenas D, Tsiamis G, Bourtzis K (2012) Bacterial diversity in Cr(VI) and Cr(III)-contaminated industrial wastewaters. Extremophiles 16(2):285–296

Kim DU, Kim MS, Lim JS, Ka JO (2013) Widespread occurrence of the tfd-II genes in soil bacteria revealed by nucleotide sequence analysis of 2,4-dichlorophenoxyacetic acid degradative plasmids pDB1 and p712. Plasmid 69(3):243–248

Kunito T, Kusano T, Oyaizu H, Senoo K, Kanazawa S, Matsumoto S (1996) Cloning and sequence analysis of *czc* genes in *Alcaligenes* sp. strain CT14. Biosci Biotechnol Biochem 60 (4):699–704

La Duc MT, Nicholson W, Kern R, Venkateswaran K (2003) Microbial characterization of the Mars Odyssey spacecraft and its encapsulation facility. Environ Microbiol 5(10):977–985

Langevin S, Vincelette J, Bekal S, Gaudreau C (2011) First case of invasive human infection caused by *Cupriavidus metallidurans*. J Clin Microbiol 49(2):744–745

Lejeune P, Mergeay M, Van Gijsegem F, Faelen M, Gerits J, Toussaint A (1983) Chromosome transfer and R-prime plasmid formation mediated by plasmid pULB113 (RP4-mini-Mu) in *Alcaligenes eutrophus* CH34 and *Pseudomonas fluorescens* 6.2. J Bacteriol 155(3):1015–1026

Li J, Zhang T, Wang L, Liu Y, Dai R, Liu X (2011) Characterization and quantification of the nickel resistant microbial community in activated sludge using 16S rDNA and nickel resistance genes. Environ Technol 32(5):533–542

Liebert CA, Hall RM, Summers AO (1999) Transposon Tn*21*, flagship of the floating genome. Microbiol Mol Biol Rev 63(3):507–522

Liesegang H, Lemke K, Siddiqui RA, Schlegel HG (1993) Characterization of the inducible nickel and cobalt resistance determinant *cnr* from pMOL28 of *Alcaligenes eutrophus* CH34. J Bacteriol 175(3):767–778

Lykidis A, Perez-Pantoja D, Ledger T, Mavromatis K, Anderson IJ, Ivanova NN, Hooper SD, Lapidus A, Lucas S, Gonzalez B, Kyrpides NC (2010) The complete multipartite genome sequence of *Cupriavidus necator* JMP134, a versatile pollutant degrader. PLoS ONE 5(3):e9729

Makkar NS, Casida LE (1987) *Cupriavidus necator* gen. nov., sp. nov.: a nonobligate bacterial predator of bacteria in soil. Int J Syst Bacteriol 37(4):323–326

Martin LJ, Quinn JE, Ellis EC, Shaw MR, Dorning MA, Hallett LM, Heller NE, Hobbs RJ, Kraft CE, Law E, Michel NL, Perring MP, Shirey PD, Wiederholt R (2014) Conservation opportunities across the world's anthromes. Divers Distrib 20(7):745–755

Martinez B, Tomkins J, Wackett LP, Wing R, Sadowsky MJ (2001) Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. J Bacteriol 183(19):5684–5697

Matus V, Sanchez MA, Martinez M, Gonzalez B (2003) Efficient degradation of 2,4,6-Trichlorophenol requires a set of catabolic genes related to tcp genes from *Ralstonia eutropha* JMP134(pJP4). Appl Environ Microbiol 69(12):7108–7115

Mergeay M (2000) Bacteria adapted to industrial biotopes: the metal resistant *Ralstonia*. In: Storz G, Hengge-Aronis R (eds) Bacterial stress responses. ASM Press, Washington DC, pp 403–414

Mergeay M, Houba C, Gerits J (1978) Extrachromosomal inheritance controlling resistance to cadmium, cobalt, copper and zinc ions: evidence from curing a *Pseudomonas*. Arch Int Physiol Biochim Biophys 86(2):440–442

Mergeay M, Monchy S, Vallaeys T, Auquier V, Benotmane A, Bertin P, Taghavi S, Dunn J, van der Lelie D, Wattiez R (2003) *Ralstonia metallidurans*, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes. FEMS Microbiol Rev 27(2–3):385–410

Mergeay M, Nies D, Schlegel HG, Gerits J, Charles P, Van Gijsegem F (1985) *Alcaligenes eutrophus* CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. J Bacteriol 162(1):328–334

Mijnendonckx K, Provoost A, Ott CM, Venkateswaran K, Mahillon J, Leys N, Van Houdt R (2013) Characterization of the survival ability of *Cupriavidus metallidurans* and *Ralstonia pickettii* from space-related environments. Microb Ecol 65(2):347–360

Misra TK (1992) Bacterial resistances to inorganic mercury salts and organomercurials. Plasmid 27(1):4–16

Miyake-Nakayama C, Ikatsu H, Kashihara M, Tanaka M, Arita M, Miyoshi S, Shinoda S (2006) Biodegradation of dichloromethane by the polyvinyl alcohol-immobilized methylotrophic bacterium *Ralstonia metallidurans* PD11. Appl Microbiol Biotechnol 70(5):625–630

Miyake-Nakayama C, Masujima S, Ikatsu H, Miyoshi S-I, Shinoda S (2004) Isolation and characterization of a new dichloromethane degrading bacterium, *Ralstonia metallidurans*, PD1. Biocontrol Science 9(4):89–93

Mobley HL, Chen CM, Silver S, Rosen BP (1983) Cloning and expression of R-factor mediated arsenate resistance in *Escherichia coli*. Mol Gen Genet 191(3):421–426

Monchy S, Benotmane MA, Janssen P, Vallaeys T, Taghavi S, van der Lelie D, Mergeay M (2007) Plasmids pMOL28 and pMOL30 of *Cupriavidus metallidurans* are specialized in the maximal viable response to heavy metals. J Bacteriol 189(20):7417–7425

Monsieurs P, Mijnendonckx K, Provoost A, Venkateswaran K, Ott CM, Leys N, Van Houdt R (2014) Genome sequences of *Cupriavidus metallidurans* strains NA1, NA4, and NE12, isolated from space equipment. Genome Announc 2(4):e00719–e00714

Monsieurs P, Provoost A, Mijnendonckx K, Leys N, Gaudreau C, Van Houdt R (2013) Genome sequence of *Cupriavidus metallidurans* strain H1130, isolated from an invasive human infection. Genome Announc 1(6):e01051–e01013

Moodley A, Nielsen SS, Guardabassi L (2011) Effects of tetracycline and zinc on selection of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 398 in pigs. Vet Microbiol 152(3–4):420–423

Müller K, Chadefaux C, Thomas N, Reiche I (2011) Microbial attack of archaeological bones versus high concentrations of heavy metals in the burial environment. A case study of animal bones from a mediaeval copper workshop in Paris. Palaeogeogr Palaeoclimatol Palaeoecol 310(1–2):39–51

Nakahara H, Ishikawa T, Sarai Y, Kondo I, Kozukue H (1977) Mercury resistance and R plasmids in *Escherichia coli* isolated from clinical lesions in Japan. Antimicrob Agents Chemother 11(6):999–1003

Newcombe DA, La Duc MT, Vaishampayan P, Venkateswaran K (2008) Impact of assembly, testing and launch operations on the airborne bacterial diversity within a spacecraft assembly facility clean-room. Int J Astrobiol 7(3 & 4):223–236

Ni'Bhriain NN, Silver S, Foster TJ (1983) Tn*5* insertion mutations in the mercuric ion resistance genes derived from plasmid R100. J Bacteriol 155(2):690–703

Nies A, Nies DH, Silver S (1989) Cloning and expression of plasmid genes encoding resistances to chromate and cobalt in *Alcaligenes eutrophus*. J Bacteriol 171(9):5065–5070

Nies D, Mergeay M, Friedrich B, Schlegel HG (1987) Cloning of plasmid genes encoding resistance to cadmium, zinc, and cobalt in *Alcaligenes eutrophus* CH34. J Bacteriol 169(10):4865–4868

Nies DH (1995) The cobalt, zinc, and cadmium efflux system CzcABC from *Alcaligenes eutrophus* functions as a cation-proton antiporter in *Escherichia coli*. J Bacteriol 177(10):2707–2712

Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51(6):730–750

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev 27(2–3):313–339

Nies DH, Koch S, Wachi S, Peitzsch N, Saier MH Jr (1998) CHR, a novel family of prokaryotic proton motive force-driven transporters probably containing chromate/sulfate antiporters. J Bacteriol 180(21):5799–5802

Nies DH, Silver S (1995) Ion efflux systems involved in bacterial metal resistances. J Ind Microbiol 14(2):186–199

Nojiri H, Tsuda M, Fukuda M, Kamagata Y (2014) Biodegradative bacteria: how bacteria degrade, survive, adapt, and evolve. Springer, Japan

Novick RP, Roth C (1968) Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. J Bacteriol 95(4):1335–1342

Palleroni NJ (2003) Prokaryote taxonomy of the 20th century and the impact of studies on the genus *Pseudomonas*: a personal view. Microbiology 149(Pt 1):1–7

Peitzsch N, Eberz G, Nies DH (1998) *Alcaligenes eutrophus* as a bacterial chromate sensor. Appl Environ Microbiol 64(2):453–458

Perez-Pantoja D, De la Iglesia R, Pieper DH, Gonzalez B (2008) Metabolic reconstruction of aromatic compounds degradation from the genome of the amazing pollutant-degrading bacterium *Cupriavidus necator* JMP134. FEMS Microbiol Rev 32(5):736–794

Perez-Pantoja D, Donoso RA, Sanchez MA, Gonzalez B (2009) Genuine genetic redundancy in maleylacetate-reductase-encoding genes involved in degradation of haloaromatic compounds by *Cupriavidus necator* JMP134. Microbiology 155(Pt 11):3641–3651

Peyru G, Wexler LF, Novick RP (1969) Naturally occurring penicillinase plasmids in *Staphylococcus aureus*. J Bacteriol 98(1):215–221

Pohlmann A, Fricke WF, Reinecke F, Kusian B, Liesegang H, Cramm R, Eitinger T, Ewering C, Potter M, Schwartz E, Strittmatter A, Voss I, Gottschalk G, Steinbuchel A, Friedrich B, Bowien B (2006) Genome sequence of the bioplastic-producing "Knallgas" bacterium *Ralstonia eutropha* H16. Nat Biotechnol 24(10):1257–1262

Pumpel T, Macaskie LE, Finlay JA, Diels L, Tsezos M (2003) Nickel removal from nickel plating waste water using a biologically active moving-bed sand filter. Biometals 16(4):567–581

Reith F, Etschmann B, Grosse C, Moors H, Benotmane MA, Monsieurs P, Grass G, Doonan C, Vogt S, Lai B, Martinez-Criado G, George GN, Nies DH, Mergeay M, Pring A, Southam G, Brugger J (2009) Mechanisms of gold biomineralization in the bacterium *Cupriavidus metallidurans*. Proc Natl Acad Sci USA 106(42):17757–17762

Reith F, Lengke MF, Falconer D, Craw D, Southam G (2007) The geomicrobiology of gold. ISME J 1(7):567–584

Reith F, Rogers SL, McPhail DC, Webb D (2006) Biomineralization of gold: biofilms on bacterioform gold. Science 313(5784):233–236

Rousseaux S, Soulas G, Hartmann A (2002) Plasmid localisation of atrazine-degrading genes in newly described *Chelatobacter* and *Arthrobacter* strains. FEMS Microbiol Ecol 41(1):69–75

Ryan MP, Pembroke JT, Adley CC (2006) *Ralstonia pickettii*: a persistent gram-negative nosocomial infectious organism. J Hosp Infect 62(3):278–284

Ryan MP, Pembroke JT, Adley CC (2009) Novel Tn*4371*-ICE like element in *Ralstonia pickettii* and genome mining for comparative elements. BMC Microbiol 9:242

Sahl JW, Schmidt R, Swanner ED, Mandernack KW, Templeton AS, Kieft TL, Smith RL, Sanford WE, Callaghan RL, Mitton JB, Spear JR (2008) Subsurface microbial diversity in deep-granitic-fracture water in Colorado. Appl Environ Microbiol 74(1):143–152

Sato Y, Nishihara H, Yoshida M, Watanabe M, Rondal JD, Concepcion RN, Ohta H (2006) *Cupriavidus pinatubonensis* sp. nov. and *Cupriavidus laharis* sp. nov., novel hydrogen-oxidizing, facultatively chemolithotrophic bacteria isolated from volcanic mudflow deposits from Mt. Pinatubo in the Philippines. Int J Syst Evol Microbiol 56(Pt 5): 973–978

Schlegel HG, Gottschalk G, Von Bartha R (1961) Formation and utilization of poly-beta-hydroxybutyric acid by Knallgas bacteria (*Hydrogenomonas*). Nature 191:463–465

Schmidt T, Schlegel HG (1989) Nickel and cobalt resistance of various bacteria isolated from soil and highly polluted domestic and industrial wastes. FEMS Microbiol Lett 62(5):315–328

Schmidt T, Schlegel HG (1994) Combined nickel-cobalt-cadmium resistance encoded by the *ncc* locus of *Alcaligenes xylosoxidans* 31A. J Bacteriol 176(22):7045–7054

Schmidt T, Stoppel RD, Schlegel HG (1991) High-level nickel resistance in *Alcaligenes xylosoxydans* 31A and *Alcaligenes eutrophus* KTO2. Appl Environ Microbiol 57(11):3301–3309

Schwartz E, Friedrich B (2001) A physical map of the megaplasmid pHG1, one of three genomic replicons in *Ralstonia eutropha* H16. FEMS Microbiol Lett 201(2):213–219

Sensfuss C, Schlegel HG (1988) Plasmid pMOL28-encoded resistance to nickel is due to specific efflux. FEMS Microbiol Lett 55:295–298

Silver S (1996) Bacterial resistances to toxic metal ions—a review. Gene 179(1):9–19

Silver S, Misra TK (1984) Bacterial transformations of and resistances to heavy metals. Basic Life Sci 28:23–46

Silver S, Misra TK (1988) Plasmid-mediated heavy metal resistances. Annu Rev Microbiol 42:717–743

Silver S, Phung LT (1996) Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol 50:753–789

Smith K, Novick RP (1972) Genetic studies on plasmid-linked cadmium resistance in *Staphylococcus aureus*. J Bacteriol 112(2):761–772

Sohrabi A, Norouzfar ZS, Eslamifar A, Arashkia A, Azadmanesh K (2011) Isolation of *Cupriavidus metallidurans* from razor blade during paraffin embedded tissue sectioning. Clin Lab 57(7–8):641

Sota M, Yano H, Nagata Y, Ohtsubo Y, Genka H, Anbutsu H, Kawasaki H, Tsuda M (2006) Functional analysis of unique class II insertion sequence IS*1071*. Appl Environ Microbiol 72(1):291–297

Springael D, Diels L, Mergeay M (1994) Transfer and expression of PCB-degradative genes into heavy metal resistant *Alcaligenes eutrophus* strains. Biodegradation 5(3–4):343–357

Springael D, Kreps S, Mergeay M (1993) Identification of a catabolic transposon, Tn*4371*, carrying biphenyl and 4-chlorobiphenyl degradation genes in *Alcaligenes eutrophus* A5. J Bacteriol 175(6):1674–1681

Stamper DM, Radosevich M, Hallberg KB, Traina SJ, Tuovinen OH (2002) *Ralstonia basilensis* M91-3, a denitrifying soil bacterium capable of using s-triazines as nitrogen sources. Can J Microbiol 48(12):1089–1098

Stanier RY, Palleroni NJ, Doudoroff M (1966) The aerobic pseudomonads: a taxonomic study. J Gen Microbiol 43(2):159–271

Stoppel RD, Meyer M, Schlegel HG (1995) The nickel resistance determinant cloned from the Enterobacterium *Klebsiella oxytoca*—conjugational transfer, expression, regulation and DNA homologies to various nickel-resistant bacteria. Biometals 8(1):70–79

Stoppel RD, Schlegel HG (1995) Nickel-resistant bacteria from anthropogenically nickel-polluted and naturally nickel-percolated ecosystems. Appl Environ Microbiol 61(6):2276–2285

Stovall SH, Wisdom C, McKamie W, Ware W, Dedman H, Fiser RT (2010) Nosocomial transmission of *Cupriavidus pauculus* during extracorporeal membrane oxygenation. ASAIO J 56(5):486–487

Summers AO, Silver S (1972) Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. J Bacteriol 112(3):1228–1236

Summers AO, Silver S (1978) Microbial transformations of metals. Annu Rev Microbiol 32:637–672

Tetaz TJ, Luke RK (1983) Plasmid-controlled resistance to copper in *Escherichia coli*. J Bacteriol 154(3):1263–1268

Timotius K, Schlegel HG (1987) Aus Abwässern isolierte nickel-resistente Bakterien. Nachrichten der Akademie der Wissenschaften Göttingen II Math-Physik Klasse 3:15–23

Trefault N, De la Iglesia R, Molina AM, Manzano M, Ledger T, Perez-Pantoja D, Sanchez MA, Stuardo M, Gonzalez B (2004) Genetic organization of the catabolic plasmid pJP4 from *Ralstonia eutropha* JMP134 (pJP4) reveals mechanisms of adaptation to chloroaromatic pollutants and evolution of specialized chloroaromatic degradation pathways. Environ Microbiol 6(7):655–668

Van Houdt R, Monchy S, Leys N, Mergeay M (2009) New mobile genetic elements in *Cupriavidus metallidurans* CH34, their possible roles and occurrence in other bacteria. Anton Leeuw Int J G 96:205–226

Van Houdt R, Monsieurs P, Mijnendonckx K, Provoost A, Janssen A, Mergeay M, Leys N (2012) Variation in genomic islands contribute to genome plasticity in *Cupriavidus metallidurans*. BMC Genom 13:111

Vandamme P, Coenye T (2004) Taxonomy of the genus *Cupriavidus*: a tale of lost and found. Int J Syst Evol Microbiol 54(Pt 6):2285–2289

Vaneechoutte M, Kampfer P, De Baere T, Falsen E, Verschraegen G (2004) *Wautersia* gen. nov., a novel genus accommodating the phylogenetic lineage including *Ralstonia eutropha* and related species, and proposal of *Ralstonia* [*Pseudomonas*] *syzygii* (Roberts et al. 1990) comb. nov. Int J Syst Evol Microbiol 54(Pt 2): 317–327

von Rozycki T, Nies DH (2009) *Cupriavidus metallidurans*: evolution of a metal-resistant bacterium. Anton Leeuw Int J G 96(2):115–139

Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y (1995) Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. Nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. Nov., *Ralstonia solanacearum* (Smith 1896) comb. Nov. and *Ralstonia eutropha* (Davis 1969) comb. Nov. Microbiol Immunol 39(11): 897–904

Zhao X-Q, Wang R-C, Lu X-C, Lu J-J, Li J, Hu H (2012) Tolerance and biosorption of heavy metals by *Cupriavidus metallidurans* strain XXKD-1 isolated from a subsurface laneway in the Qixiashan Pb-Zn sulfide Minery in eastern China. Geomicrobiol J 29(3):274–286

Chapter 2
Genomic Context of Metal Response Genes in *Cupriavidus metallidurans* with a Focus on Strain CH34

Rob Van Houdt and Max Mergeay

Abstract *Cupriavidus metallidurans* CH34 has been studied for over 30 years, mostly because of its resistance to numerous heavy metals. Many of these metal resistance determinants were rapidly associated with native megaplasmids. However, its genome sequencing and whole genome expression profiling not only revealed the complex structure of its multiple replicons and complex responses to metals, but also revealed the presence of unnoticed/unstudied metal resistance determinants on the different replicons. In this chapter, the genomic context of the metal response genes in *C. metallidurans* CH34 will be described with a focus on its mobilome including insertion sequence elements, transposons, integrative and conjugative elements and genomic islands.

Keywords *Cupriavidus metallidurans* · Megaplasmids · Chromid · Mobile genetic elements · Metal resistance · Adaptation

2.1 A Genome with Multiple Replicons

All *Cupriavidus* and *Ralstonia* genomes are typified by the presence of a large replicon in addition to their chromosome and megaplasmids. These large replicons with a size of around 2–3 Mb have been designated both 'second or secondary chromosomes' as well as 'megaplasmids'. The ambiguity in their nomenclature arises from the chimeric features of these replicons since they carry essential genes making them indispensable for cell viability (second chromosome) and they use a plasmid-type replication system rather than a chromosomal one (megaplasmids). To distinguish this particular replicon, which does not completely fit the term

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chromosome or plasmid, Harrison and colleagues (2010) coined the term "chromid". Three main criteria were put forward in defining these replicons: (i) chromids have plasmid-type maintenance and replication systems, (ii) chromids have a nucleotide composition similar to that of the chromosome and (iii) chromids carry core genes that are found on the chromosome in other species (Harrison et al. 2010). However, the presence of chromids is not limited to these genera or to the β-proteobacteria since chromids have also been observed in γ-proteobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, *Deinococcus-Thermus*, Firmicutes and Spirochaetes (Harrison et al. 2010; Van Houdt and Mergeay 2012).

Most *Cupriavidus* and *Ralstonia* strains carry one or more megaplasmids with a size of 100 kb or larger (for a detailed review see Schwartz 2009). Acquiring these megaplasmids was probably key to the adaptation of the strains to certain ecological niches, considering the distinct and specialized functions contained within the plasmids. For instance, pMOL28 and pMOL30 from *C. metallidurans* CH34 are both involved in heavy metal resistance, pHG1 of *C. eutrophus* H16 is involved in hydrogenotrophy and chemolithotrophy (Schwartz et al. 2003; Schwartz 2009), while pRALTA of *C. taiwanensis* LMG19424 carries nitrogen fixation and legume symbiosis functions (Amadou et al. 2008). In addition to the megaplasmids, other plasmids can be present, most of which are broad host range (BHR) plasmids such as the IncP-1β plasmid pJP4 from *C. pinatubonensis* JMP134 (*R. eutropha* JMP134) (Don and Pemberton 1981, 1985; Lykidis et al. 2010; Sato et al. 2006) and the PromA plasmids captured by *C. metallidurans* CH34 (Top et al. 1994; Van der Auwera et al. 2009; Gstalder et al. 2003; Mela et al. 2008; Tauch et al. 2002; van Elsas et al. 1998).

This chapter mainly focuses on the genomic context of *C. metallidurans* type strain CH34 in close comparison with other *C. metallidurans* strains (Table 2.1). DNA-DNA hybridization (DDH) was estimated via the Genome-to-Genome Distance Calculator (Meier-Kolthoff et al. 2013) to confirm the taxonomic position of recently isolated and sequenced *Cupriavidus* strains. This analysis indicated that *Cupriavidus* sp. HMR-1 belongs to the *C. metallidurans* species (DDH value of 72.2 %), while *Cupriavidus* sp. BIS7 (Hong et al. 2012) does not (DDH value of 30.0 %). In addition, the available genomes of other *Cupriavidus* species, *Ralstonia* species and other closely related β-proteobacterial genomes were taken into account for a comprehensive analysis. These include *C. pinatubonensis* JMP134 (Lykidis et al. 2010), *C. eutrophus* H16 (*C. necator* H16, *R. eutropha* H16) (Fricke et al. 2009), *C. necator* N-1 (Poehlein et al. 2012), *C. taiwanensis* LMG19424 (Amadou et al. 2008), *R. solanacearum* species complex (Salanoubat et al. 2002; Remenant et al. 2011), and *R. pickettii* strains 12J and 12D (Yang et al. 2010). Other β-proteobacterial genomes included are *Delftia acidovorans* SPH-1, *Comamonas testosteroni* KF-1 and *Bordetella petrii* DSM12804. The latter strains only have one chromosome of ~6 Mb, in contrast to the various replicons found in *Cupriavidus* strains, although totalling a comparable amount of genetic material.

Table 2.1 *C. metallidurans* strains compared in this study

Strain	Isolation site	Isolation place	References
31A	Galvanization tank, metal factory	Holzminden, Germany	Schmidt et al. (1991)
43015	Human cerebrospinal fluid	Göteborg, Sweden	CCUG[a]
45957	Pharmaceutical industry	Sweden	CCUG
AS167	Mine tailings	Likasi-Sud, Congo	Brim et al. (1999)
AS168	Mine tailings	Likasi-Sud, Congo	Diels and Mergeay (1990)
AS39	Mine tailings	Likasi-Sud, Congo	Diels and Mergeay (1990)
CH34[T*]	Decantation tank, zinc factory	Liège, Belgium	Janssen et al. (2010)
CH42	Polluted sediments, zinc factory	Liège, Belgium	Brim et al. (1999)
CH79	Polluted sediments, zinc factory	Liège, Belgium	Brim et al. (1999)
H1130[*]	Patient with nosocomial septicaemia	Québec, Canada	Monsieurs et al. (2013)
HMR-1[*]	Wastewater treatment plant	Hong Kong, China	Li et al. (2013)
KT01	Wastewater treatment plant	Göttingen, Germany	Timotius and Schlegel (1987)
KT02	Wastewater treatment plant	Göttingen, Germany	Schmidt et al. (1991)
KT21	Wastewater treatment plant	Göttingen, Germany	Timotius and Schlegel (1987)
NA1[*]	Water storage system	ISS[b]	Monsieurs et al. (2014)
NA2	Contingency water container	ISS	Mijnendonckx et al. (2013)
NA4[*]	Water recovery system	ISS	Monsieurs et al. (2014)
NE12[*]	Cleanroom Kennedy Space Center	Florida, USA	Monsieurs et al. (2014)
SV661	Non-ferrous industry	Beerse, Belgium	Diels and Mergeay (1990)

[*]Genome sequence available
[a]Culture Collection, University of Göteborg, Sweden
[b]International Space Station

2.2 The Megaplasmids pMOL28 and pMOL30

Like *C. metallidurans* CH34, most of the related bacteria isolated from metal-rich biotopes carry megaplasmids conferring high level resistance to heavy metals. The size of these megaplasmids ranges from 180 to 450 kb and some of them are conjugative with much higher transfer frequencies than those found in CH34, based

on zinc, cobalt or cadmium as selective marker (Diels et al. 1989, 1995). However, up to now, only pMOL28 and pMOL30 from *C. metallidurans* CH34 have been sequenced (fully closed), thoroughly annotated and studied at the transcriptomic level after various challenges by heavy metals (Janssen et al. 2010; Monchy et al. 2006a).

Plasmid pMOL28 (171 kb) confers resistance to nickel and cobalt (Liesegang et al. 1993; Mergeay et al. 1985), chromate (Nies et al. 1990) and mercury (Diels et al. 1985). It is self-transferred at low frequency (Diels et al. 1989), although it contains an almost complete conjugative gene set similar to that carried by megaplasmid pHG1 from *C. eutrophus* H16, which is self-transferrable at high frequency (Friedrich et al. 1981). The conjugative transfer of pMOL28 can be enhanced through co-transfer with the BHR IncP-1α plasmid RP4. This boosted transfer is assisted through transposition of Tn*4378* (see Sect. 2.4.2), cointegrate formation and resolution. In addition, pMOL50, a derivative of pMOL28 obtained from a survivor of growth at 37 °C, was found to be highly conjugative and was used to make a circular genetic map of the *C. metallidurans* CH34 chromosome (Sadouk and Mergeay 1993). It was not perceived at that time that this map did not include the genes now known to be carried by the chromid.

Two distinct conserved synteny blocks are shared by pMOL28, plasmids carried by other *C. metallidurans* strains such as NA1, NA4, NE12 and HMR-1, pHG1 from *C. eutrophus* H16 (Schwartz et al. 2003), pBB2 from *C. necator* N-1 (Poehlein et al. 2012), and to a lesser extent pRALTA from *C. taiwanensis* LMG19424 (Amadou et al. 2008) and pBVIE02 from *Burkholderia vietnamiensis* G4. In pMOL28, and likely also for similar plasmids from other *C. metallidurans* strains, these synteny blocks are separated by genomic islands (GIs) that group all genes involved in heavy metal resistance (Mergeay et al. 2009). This indicates that related β-proteobacteria carry plasmids with similar backbone functions (replication, maintenance and transfer) but with additional and different accessory genes contributing to the adaptation to their specific niches. The backbone similarity between pMOL28 and pHG1, pRALTA or pBB2 is quite variable but low for *parAB* and *repA* suggesting plasmid compatibility.

Plasmid pMOL30 (234 kb) confers resistance to mercury (Diels et al. 1985), zinc, cadmium and cobalt (Mergeay et al. 1985; Nies 1995), lead (Borremans et al. 2001), silver (Bersch et al. 2011), and copper (Collard et al. 1994). It is not self-transmissible but it can be mobilized at very low transfer frequency probably with the aid of conjugation genes located on other replicons. Comparable with pMOL28, its conjugative transfer can also be enhanced through co-transfer with the BHR plasmid RP4 via transposition of Tn*4380* (see Sect. 2.4.2). All pMOL30 genes related to heavy metal resistance are also located on genomic islands, while the backbone is only shared with plasmids from other *C. metallidurans* strains such as NA1, NA4 and NE12 and to lesser extent with pBVIE01 of *B. vietnamiensis* G4 (Mergeay et al. 2009).

Recently, *C. metallidurans* strains from different sites, ranging from the pharmaceutical and space industry to metal mining and metal industries, waste treatment plants and even human infection, have all (except one) been shown to carry at least

one megaplasmid (Mijnendonckx et al. 2013; Van Houdt et al. 2012b). For all strains, comparative genome hybridization (CGH) showed a strong conservation of the pMOL28 and pMOL30 GIs and their accessory genes related to metal resistance, while more variation was observed for the backbones (Van Houdt et al. 2012b). For instance strain 31A, which carries the two megaplasmids pTOM8 and pTOM9 (Schmidt and Schlegel 1989), showed only a positive hybridization signal with the pMOL28 backbone, indicating that one of the plasmids has a backbone related to pMOL28, while the other has a backbone unrelated to pMOL30 (Van Houdt et al. 2012b). These observations extend the biotopes where *C. metallidurans* hosts that carry typical metal resistance plasmids are found beyond just metal-contaminated sites. Therefore, these resistance determinants could be acquired earlier in evolution, which would be consistent with the hypothesis that toxic metal resistance systems pre-existed the recent anthropogenic activities and arose soon after life began, in a world already polluted by volcanic activities (Silver and Phung 1996). However, taking into account that most of the metal determinants are on the native megaplasmids and the GIs thereon, it could be argued that anthropogenic activities and industrially polluted environments provided a selective pressure for the conservation of these determinants or even the acquisition of more, considering both the arsenal of determinants as well as the level of resistance.

2.3 The *C. metallidurans* CH34 Chromid: A Diversified Reservoir of Metal Response Genes

As previously mentioned, all *Cupriavidus* and *Ralstonia* genomes are typified by the presence of an additional replicon designated as the chromid. For *Cupriavidus* chromids, the gene cluster *csp repA csp parA parB xerD*, which is involved in replication and partitioning, is strongly conserved. The *repA* upstream region contains 3 putative DnaA boxes and many repetitive 17 nt-long elements with a highly conserved motif, which may be RepA binding sites (Janssen et al. 2010). The partition proteins belong to the ParAB superfamily, as is the case for most chromids, and these Par proteins tend to phylogenetically group with plasmid-encoded Par proteins rather than those encoded by primary chromosomes (Dubarry et al. 2006). Analysis of the similarity between and phylogenetic relationships of the chromid-encoded ParA and ParB proteins from 19 strains belonging to the *Cupriavidus/Ralstonia* and *Burkholderia* lineages, indicates that two distinct plasmids may have been the origin of the chromids present in the genera *Cupriavidus/Ralstonia* and *Burkholderia* (Lykidis et al. 2010).

The majority (7 out of 12) of the RND (Resistance-Nodulation- Division)-driven efflux systems of *C. metallidurans* CH34 are located on the chromid. Most of these systems still remain to be fully elucidated like *cusDCBAF*, *zniBAC* and *zneBAC*, while others are most likely inactive (*nimBAC* inactivated via insertion of IS*Rme3*

in *nimA*; *hmyCBA*, insertion of IS*1088* into *hmyA*; *hmvCBA*, nonsense mutation in *hmv*A; $czcC_2B_2A_2$, insertion of Tn*6050* in $czcB_2$). It could be that the chromid allowed adaptation to environments with moderate heavy metal concentrations, for instance in a (volcanic) biotope colonized by an ancestor of CH34. The formation and acquisition of the megaplasmids pMOL28 and pMOL30 may well be key to surviving high metal concentrations in industrial biotopes, and could putatively have led to the inactivation of redundant genes.

Two particular RND loci on the CH34 chromid attract attention. The first locus codes, in addition to the RND system ($czcI_2C_2B_2A_2$), also for an ATPase (*zntA*). It is part of a larger conserved synteny block in (at least) CH34, H1130, NA4 and NE12, indicating that it is part of the chromid core. The RND locus is separated from its divergently transcribed cognate two-component regulatory system *czcRS* by *ubiG* coding for a 3-demethylubiquinone-9 3-methyltransferase. The latter participates in the biosynthesis of ubiquinone, a mobile redox carrier in the respiratory chain. In *Escherichia coli*, ubiquinone and demethylmenaquinone have been shown to affect the ArcB sensor kinase activity of the two-component system AcrBA (Sharma et al. 2013; Alvarez et al. 2013). Further downstream, the *czcL*, *hns* and *mmmQ* genes are present, coding for an unknown protein, an H-NS like protein and a small stress responsive protein, respectively. The $CzcC_2B_2A_2$ efflux pump is highly identical (60–80 %) to CzcCBA encoded by pMOL30, suggesting efflux of similar divalent cations (Cd^{2+}, Zn^{2+} and Co^{2+}). In CH34, however, $czcB_2$ is inactivated by Tn*6050* insertion and $czcI_2C_2B_2'$ is separated from $czcB_2''A_2$ through a large chromosomal inversion via homologous recombination with a second Tn*6050* copy (Van Houdt et al. 2009). The efflux pump appears to be intact in all other strains.

The second locus holds two RND systems and the uncharacterized membrane-anchored protein ZnfP (Fig. 2.1). Again the locus is part of a larger conserved synteny block in (at least) CH34, H1130, NA4 and NE12, indicating that

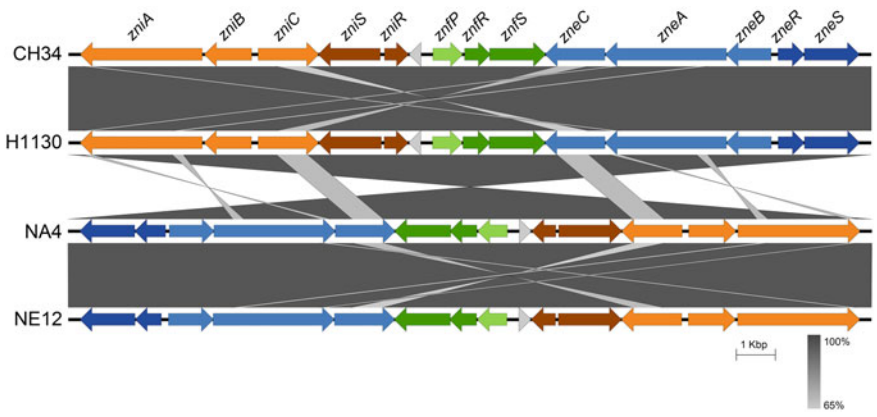

Fig. 2.1 Map showing the genomic organization of two RND systems on the chromid of *C. metallidurans* CH34, H1130, NA4 and NE12. Cognate two-component regulatory systems are coloured darker than their proposed regulon. The *grey scale* indicates the levels of synteny

it is part of the chromid core. The *zne* cluster is likely linked to zinc resistance (see volume II) and has a *BAC* gene order instead of the more common *CBA*. Also the *zniABCSR* cluster has an uncharacteristic structure, with both the efflux pump as well as the regulatory system coding genes being divergently transcribed (Fig. 2.1). Although *zne* and *zni* systems with similar gene order arrangements are found in many *Ralstonia* spp., they are not present in other *Cupriavidus* species. Next to the RND-driven efflux systems, the chromid also carries a cluster of genes involved in chromate resistance and one involved in copper resistance. Both have a more extensive counterpart on pMOL28 and pMOL30, respectively (see Chap. 3). The chromid-located cluster involved in chromate resistance codes for a chromate efflux pump $ChrA_2$ (Rmet_3865), a transcriptional repressor $ChrF_2$ (Rmet_3864), and a putative transcriptional activator $ChrB_2$ (Rmet_3866) (Juhnke et al. 2002). Both the chromid- and pMOL28-encoded efflux pump belong to the CHR2 subgroup (Diaz-Perez et al. 2007) of the CHR transporter family (first described by Nies et al. 1998). The locus is located on a highly conserved synteny block (>160 kb) shared by CH34, NA4 and H1130. Strains NA1, NA2 and HMR-1 also share this highly conserved synteny block, but not the chromate cluster. The chromid-located basic $copS_2R_2A_2B_2C_2D_2$ encodes for homologs of the Cop/Pco system responsible for the handling of periplasmic copper (Bondarczuk and Piotrowska-Seget 2013).

2.4 Mobile Genetic Elements and Genomic Islands in *C. metallidurans* CH34

2.4.1 Genomic Islands

The *C. metallidurans* CH34 genome harbours at least 22 GIs, which were identified via a composite set of criteria (Van Houdt et al. 2009; Mergeay et al. 2009) and CGH (Van Houdt et al. 2012b). Thirteen GIs were identified on the chromosome, four on the chromid, three on pMOL28 and two on pMOL30 (Table 2.2). The available information on these GIs, which was detailed in previous studies (Van Houdt et al. 2009, 2012b; Mergeay et al. 2009), will be summarized and updated below.

2.4.1.1 GIs on the CH34 Chromosome

The largest island (109 kb) on the chromosome, CMGI-1, is almost identical (>99.95 % DNA sequence similarity) to PAGI-2 isolated from the pathogenic *Pseudomonas aeruginosa* strain clone C (Klockgether et al. 2007; Larbig et al. 2002). However, the integrase gene of CMGI-1 is inactivated by Tn*6049*. CMGI-1 and PAGI-2 do not carry any specific catabolic functions but encodes proteins for the complexation and transport of heavy metals. Both belong to the large

Table 2.2 Genomic islands identified in *C. metallidurans* CH34

Element (size kb)	Location (Rmet_)	Features
CMGI-1 (109.6)	CHR (2287-2408)	Identical to island PAGI-2C of *Pseudomonas aeruginosa* clone C, TBSSR[a] inactivated by Tn*6049*
CMGI-2 (101.6)	CHR (1236-1351)	Tn*4371* ICE family (ICE_{Tn4371}6054), involved in hydrogenotrophy and degradation of aromatic compounds
CMGI-3 (97.0)	CHR (1465-1560)	Tn*4371* ICE family (ICE_{Tn4371}6055), involved in hydrogenotrophy and CO_2 fixation
CMGI-4 (56.5)	CHR (2987-3045)	Tn*4371* ICE family (ΔICE_{Tn4371}6055), partial element
CMGI-5 (25.4)	CHR (2824-2847)	Remnant of integrated plasmid
CMGI-6 (17.6)	CHR (1997-2020)	TBSSR, no defined function for accessory genes
CMGI-7 (11.0)[b]	CHR (0316-0326)	TBSSR is part of a four-gene module (PRQ)
CMGI-8 (12.3)	CHR (2549-2561)	TBSSR, no defined function for accessory genes
CMGI-9 (20.6)	CHR (2156-2172)	TBSSR, no defined function for accessory genes
CMGI-10 (21.0)	CHR (3347-3368)	No defined function for accessory genes
CMGI-11 (10.8)	CHR (1660-1668)	Putative fimbrial operon, flanked by two IS elements (IS*Rme7*)
CMGI-12 (9.1)	CHR (2662-2670)	No defined function for accessory genes
CMGI-13 (15.9)	CHR (2723-2737)	Genes involved in polysaccharide biosynthesis
CMGI-A (87.1)	Chromid (4428-4305)	No defined function for accessory genes
CMGI-B+D (160.7)	Chromid (4475-4496) Chromid (4598-4715)	Multiple genes coding for phage-related proteins (associated to phages of *R. solanacearum*)
CMGI-C (7.1)	Chromid (4450-4556)	TBSSR fragment, gene coding for mannose-binding lectin
CMGI-E (120.2)	Chromid (5454-5568)	Tn*7*-related genes at one extremity, genes putatively involved in degradation of aromatic compounds
CMGI-28a (45.9)	pMOL28 (6212-6333)	heavy metal resistance genes (*mer*, *cnr* and *chr*), flanked by IS*1071* elements
CMGI-28b (23.0)	pMOL28 (6252-6263)	TBSSR is part of a four-gene module (PRQ), three *rhs*-like genes
CMGI-28c (15.0)	pMOL28 (6320-6332)	TBSSR, no defined function for accessory genes

(continued)

Table 2.2 (continued)

Element (size kb)	Location (Rmet_)	Features
CMGI-30a (74.4)	pMOL30 (6002-6171)	TBSSR, heavy metal resistance genes (*mer*, *czc* and *pbr*) flanked by Tn*4380* elements (one intact and one truncated copy)
CMGI-30b (88.0)	pMOL30 (6153-6067)	TBSSR, heavy metal resistance genes (*sil*, *ncc-nre*, and *cop*)

[a]TBSSR: tyrosine-based site-specific recombinase
[b]Updated compared to previous studies

pKLC102/PAGI-2 family of elements that share a core gene set (Miyazaki et al. 2013; Klockgether et al. 2007) and are integrated downstream of tRNA genes (Larbig et al. 2002). Self-transfer of PAGI-2 has not been detected so far, in contrast to the *clc* element of *Pseudomonas knackmussii* B13, the best studied of this family, which enables the metabolisation of chlorocatechols (Miyazaki et al. 2013). Accordingly, most of the PAGI-2C genes are transcriptionally silent in *P. aeruginosa* clone C (Klockgether et al. 2008). CMGI-1 carries the $pbrR_2$ *cadA* $pbrC_2$ cluster involved in cadmium, zinc and lead resistance (see Chap. 3) (Monchy et al. 2006b, 2007; Monsieurs et al. 2011; Legatzki et al. 2003). Through the analysis of conserved synteny blocks, the latter cluster led to the identification of transposon Tn*6052* in *Burkholderia xenovorans* LB400 (recently re-evaluated as *Paraburkholderia xenovorans*; Sawana et al. 2014), which carries eight copies of this transposon harbouring the accessory genes *pbrR cdfX pbrC*, with *cdfX* coding for a putative metal-efflux system that is distantly related to cation diffusion factors (Van Houdt et al. 2009). Thus, it provides a new mobile combination of metal-processing genes. This is one of the examples for which the analysis of synteny blocks reveals the presence of a mobile genetic element (MGE), thereby uncovering the diverse variety of MGEs from environmental β-proteobacteria.

Three of the chromosomally-located *C. metallidurans* CH34 GIs (CMGI-2, CMGI-3 and CMGI-4) belong to the Tn*4371* ICE family (Van Houdt et al. 2013, 2009; Toussaint et al. 2003). This family harbours MGEs with a quadripartite structure consisting of three core sets involved in integration (via a tyrosine-based site-specific recombinase (TBSSR)), maintenance/stability and conjugation, and one variable set containing accessory genes with diverse functions including xenobiotic catabolism, heavy metal resistance, antibiotic resistance and chemolithotrophic metabolism. The accessory genes are typically located between the conjugative genes *rlxS* (*virD2*) and *traR* (Van Houdt et al. 2013; Ryan et al. 2009; Toussaint et al. 2003; Merlin et al. 1999). Currently, this ICE_{Tn4371} family harbours more than 40 elements residing in β- and γ-proteobacteria with sizes ranging from 38 to 101 kb. CMGI-2 (ICE_{Tn4371}6054) harbours accessory genes involved in the degradation of aromatic compounds as well as in hydrogen oxidation. CMGI-3 (ICE_{Tn4371}6055) harbours genes involved in carbon dioxide fixation (Calvin-Benson-Bassham cycle) and hydrogen oxidation. Therefore, the ability of CH34 to degrade toluene or to

grow on hydrogen gas and carbon dioxide are enabled by CMGI-2 and CMGI-3. The third GI from this family, CMGI-4 ($\Delta ICE_{Tn4371}6056$), is only a partial element.

As mentioned in previous studies, CMGI-2 harbours two RIT (recombinase in trio) elements consisting of three adjacent and unidirectional overlapping genes encoding tyrosine-based site-specific recombinases (Van Houdt et al. 2009). The three TBSSRs in these RIT elements are associated with distinct TBSSR subfamilies and the elements were later found to be much more widespread (Van Houdt et al. 2012a; Ricker et al. 2013).

The contribution of the remaining chromosomally-located GIs to particular CH34 functions is unclear (Table 2.2). Although the cluster involved in arsenic resistance (Rmet_0327-Rmet_0334) was initially attributed to CMGI-7, this becomes less evident when compared with recent *C. metallidurans* genome sequences. The latter indicates that the arsenic resistance cluster is part of a conserved synteny block in all *C. metallidurans* strains (chromosomal backbone) irrespective of the presence of other CMGI-7 genes. Therefore, CMGI-7 probably only contains the Rmet_0316-Rmet_0326 region, which includes transposon Tn*6049* (in case of CH34) and a PRQ module in addition to two conserved genes coding for unknown functions. The PRQ term was used to label a synteny module coding for three putative TBSSRs and a conserved hypothetical protein. In fact, two different PRQ modules, which share very little sequence identity, were found in strain CH34. The second one is associated with genomic island CMGI-28b in plasmid pMOL28. Both share synteny with gene blocks conserved in other bacterial genomes.

2.4.1.2 GIs on the CH34 Chromid and on Megaplasmids pMOL28 and pMOL30

At least five GIs were identified on the *C. metallidurans* CH34 chromid (Table 2.2) (Van Houdt et al. 2012b). One island, CMGI-B+D, carries multiple genes coding for phage-related proteins. CMGI-E carries genes putatively involved in the degradation of aromatic compounds (vanillate O-demethylase oxygenase and phthalate 4,5-dioxygenase) (Van Houdt et al. 2012b).

As indicated in Sect. 2.2, plasmid pMOL28 and pMOL30 contain large genomic islands that harbour all plasmid-borne genes involved in the response to heavy metals. Plasmid pMOL30 carries CMGI-30a and -30b that convey resistance to cadmium, zinc, cobalt, lead and mercury, and copper and silver, respectively. Within CMGI-30a, a 25-gene cluster is highly conserved (>99 % nucleotide similarity) in all *C. metallidurans* strains (Van Houdt et al. 2012b) and in other strains like *R. pickettii* 12J (Fig. 2.2). This cluster contains next to the *czc* cluster, genes involved in membrane-related functions with *gtrM1* (Rmet_5966) and *gtrB1* (Rmet_5968) encoding for a glycosyl transferase, *gtrA1* (Rmet_5967) for a bactoprenol-linked glucose translocase, *ompP* (Rmet_5974) for a porin predicted to form a channel for the diffusion of small hydrophilic molecules, and *flgB* (Rmet_5971) for a flagellar basal body rod protein. Furthermore, this cluster is flanked by a TBSSR from a distinct family (Van Houdt et al. 2012a), which is

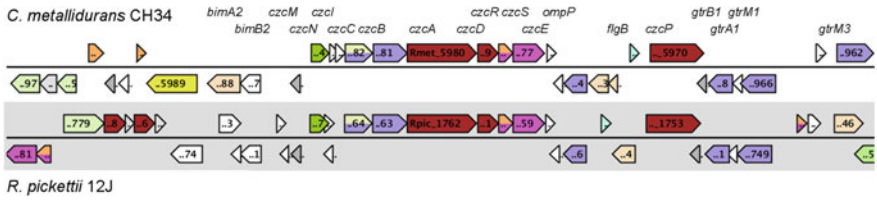

Fig. 2.2 Genomic context of the *czc* cluster on pMOL30 from *C. metallidurans* CH34 and in *R. pickettii* 12J constructed with MGcV (Overmars et al. 2013). Genes are coloured based on COG assignment (Overmars et al. 2013)

always associated with a second conserved protein of unknown function (putative transcriptional repressor) making up a bipartite module (BIM) (Van Houdt et al. 2009, 2012a). CMGI-30b contains a large cluster of 32 genes induced by copper (Monchy et al. 2006a, 2007; Monsieurs et al. 2011; Mergeay et al. 2009), which is unique with respect to its complexity compared to other copper resistance mechanisms described so far (see Chap. 3). Similar to the *czc* cluster on CMGI-30a also the *cop* cluster contains genes involved in membrane-related functions ($gtrM_2$, $gtrB_2$, $gtrA_2$ and $ompP_2$).

Three GIs were defined on plasmid pMOL28 (Table 2.2). CMGI-28a is delimited by two inactive Tn*3*-like insertion sequences (IS*1071* inactivated by Tn*6049* and IS*Rme18*) and carries all metal resistance determinants of pMOL28, thereby providing resistance to mercury, chromate and cobalt/nickel (see Chap. 3). The functions of the accessory genes of the two other GIs are unidentified. As indicated above, CMGI-28b carries the second PRQ module (Rmet_6252-6255; *prqA2B2C2R2*). In addition, it contains three genes coding for RHS-repeat-containing proteins. Rhs-like family proteins have been associated with various phenotypes such as social motility, virulence, toxin production and contact-dependent growth inhibition (Youderian and Hartzell 2007; Poole et al. 2011; Busby et al. 2013; Chen et al. 2014; Koskiniemi et al. 2013, 2014; Kung et al. 2012; Sisto et al. 2010). However, none of the three proteins carry the Rhs repeat-associated core domain (IPR022385, Hunter et al. 2012) nor the conserved motif, ending in DPXG-(18)-DPXG, that is shared by all Rhs proteins (Jackson et al. 2009).

2.4.1.3 GIs on Other *C. metallidurans* Strains

Interestingly, CGH of *C. metallidurans* strains from different sites, ranging from the pharmaceutical and space industry to metal mining and metal industries, waste treatment plants and even human infection, indicated that these strains could be divided into two main groups based on their GI content. One group carries almost all GIs identified in CH34, while these GIs were much less abundant in the second group (Van Houdt et al. 2012b) (Fig. 2.3). Although the incidence of GIs was in agreement with the functional phenotypes carried by them such as degradation of

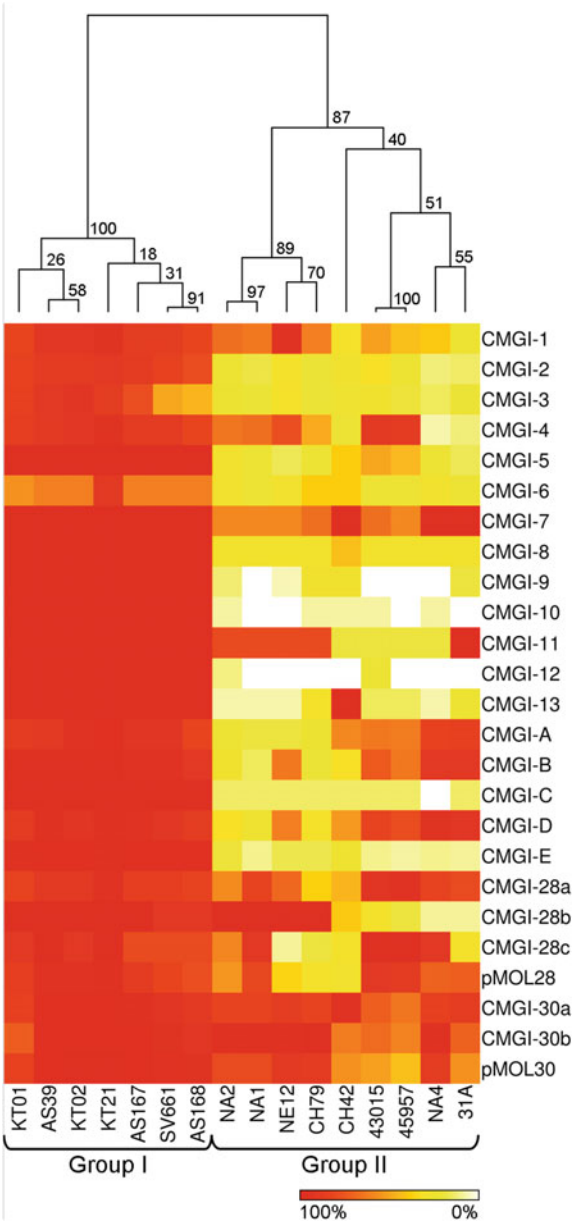

Fig. 2.3 Hierarchically (complete-linkage) clustered heat map based on CGH results related to plasmids and GIs of 16 different *C. metallidurans* strains to a whole-genome oligonucleotide DNA microarray of type strain CH34. Bootstrap values (%) from 1000 times resampling are shown at each dendrogram node (Van Houdt et al. 2012b)

toluene or the ability to grow on hydrogen gas and carbon dioxide, the isolation site characteristics and location (geographic) showed no clear correlation with the occurrence of GIs.

2.4.2 Insertion Sequence Elements and Transposons

Insertion sequence (IS) elements are small elements (typically less than 3 kb) that are capable of independent transposition and carry one or more open reading frames generally only encoding gene products essential for their mobility. They are flanked by short inverted repeat sequences and generate short directly repeated sequences of the target DNA at the point of insertion, which are 2–14 bp in size and specific for a certain element (Mahillon and Chandler 1998; Siguier et al. 2014). The CH34 genome harbours 21 distinct IS elements totalling 57 intact IS elements (Table 2.3), which are dispersed over the four replicons with most of them located on the chromosome (the largest replicon). All IS elements were categorized into 10 families, with the IS*3*, IS*30*, and IS*5* families being the most abundant. The IS*3* and IS*5* families are in fact the most abundant families among bacterial genomes (together with IS*1* and IS*481*) (Touchon and Rocha 2007; Wagner et al. 2007).

IS elements and their transposition have been implicated in the evolution of their hosts as they contribute to a variety of genomic rearrangements (deletions, inversions and duplications) that can result in gene inactivation and/or affect the expression of

Table 2.3 Distribution of IS elements in *C. metallidurans* CH34

Element	Family (sub)	Length (bp)	CHR	Chromid	pMOL28	pMOL30
IS*Rme20*	IS*21*	1977	1			
IS*Rme4*	IS*21*	2469	2			
IS*Rme9*	IS*21*	2688			1	
IS*1090*	IS*256*	1343	4			
IS*Rme11*	IS*3* (IS*150*)	1231		2		
IS*Rme12*	IS*3* (IS*150*)	1454	1			
IS*Rme17*	IS*3* (IS*150*)	1678	1			
IS*1087B*	IS*3* (IS2)	1330	2			
IS*Rme3*	IS*3* (IS*3*)	1288	3	5		2
IS*Rme15*	IS*3* (IS*51*)	1325		1		1
IS*1086*	IS*30*	1106	1	1	1	
IS*1088*	IS*30*	1103	3	6		
IS*Rme10*	IS*30*	1063				1
IS*Rme8*	IS*4*	1455	1	1		
IS*Rme5*	IS*481*	1041	3	1		
IS*Rme1*	IS*5* (IS*427*)	1331	2	2		
IS*Rme6*	IS*5* (IS*427*)	913	1			
IS*Rme7*	IS*6*	840	2			
IS*Rme19*	IS*66*	2227				1
IS*1071*	Tn*3*	3204	3		1*	
IS*Rme18*	Tn*3*	ND			1	

CHR chromosome. *ND* not determined
*Copy inactivated by Tn*6049*

adjacent genes (Bentley et al. 2008; Bickhart et al. 2009; Hubner and Hendrickson 1997; Lin et al. 2007). Therefore, they are seen as a significant force in the adaptive and evolutionary response of their host, conferring genome plasticity that allows rapid adaptation to new environments (Mira et al. 2002; Schneider and Lenski 2004).

The involvement of IS elements in adaptation has been demonstrated for a number of the IS elements in *C. metallidurans*. IS*1086* and IS*Rme1* transposed into the positive selection vectors pJV240 (Dong et al. 1992) and pGBG1 (Schneider et al. 2000). A spontaneous zinc resistant mutant of *C. metallidurans* AE126 was characterized by IS*1087* transposition into *cnrY*, which codes for an anti-sigma factor. Inactivation resulted in constitutive expression of the cobalt and nickel resistance determinant *cnr* and in increased (nonspecific) zinc efflux (Collard et al. 1993; Tibazarwa et al. 2000; Grass et al. 2000). Increased heterologous expression of the *czr* (cadmium zinc resistance) operon of *P. aeruginosa* CMG103 in *C. metallidurans* AE104, which resulted in an increased zinc resistance, was mediated by insertion of IS*1088* or IS*1090* into the *czr* promoter region (Talat 2000). Transposition of IS*1086,* IS*1087B* and IS*Rme3* was involved in increased silver resistance (Mijnendonckx et al. 2011). Finally, at least 32 ISs are inserted inside a coding sequence, thereby inactivating the gene and concurrent gene product. Examples are the insertion of IS*Rme3* in *nimA* and IS*1088* in *hmyA*, consequently affecting the efflux pumps of the Nim and Hmy heavy metal tri-component efflux systems.

Transposons differ from IS elements as they also carry accessory genes, which confer clinically or metabolically important properties on the host cell (Frost et al. 2005). The CH34 genome harbours five distinct transposon families totalling 19 intact transposons (Table 2.4), which are dispersed over the four replicons with most of them located on the chromosome (the largest replicon). The transposition modules of four transposons are related to those of mercury transposons with Tn*4378,* Tn*4380* and Tn*6050* belonging to the Tn*21*/Tn*501* family and Tn*6048* to the Tn*5053* family. The Tn*501* family transposons are typically delineated by 38 bp terminal inverted repeats, contain two genes involved in transposition (*tnpRA*), and generate 5 bp direct target DNA sequence repeats upon insertion (Brown and Evans 1991; Mindlin and Petrova 2013). The Tn*5053* family transposons are typically delineated by 25 bp terminal inverted repeats, contain four genes involved in transposition (*tniABQR*), and generate 5 bp direct repeats upon insertion (Kholodii et al. 1995; Mindlin and Petrova 2013). The latter transposons have been designated *res* site hunters as they exhibit a striking insertional preference for the *res* regions of naturally occuring plasmids (Minakhina et al. 1999). The transposition module of Tn*6049* could, at this moment, not be categorized.

Transposons Tn*4378* and Tn*4380* carry identical mercury resistance determinants (see Chap. 3) and are located on pMOL28 (in CMGI-28b) and pMOL30 (in CMGI-30a), respectively (Diels et al. 1985; Mergeay et al. 2009; Van Houdt et al. 2009). Transposon Tn*4380* is present in most of the *C. metallidurans* strains (Van Houdt et al. 2012b) and is largely recognizable (>99 % nucleotide similarity) in plasmid BRA100 and pMAB01 from the actinobacterial strains *Mycobacterium abscessus* subsp. *bolletii* F1725 and INCQS 00594 suggesting an effective

Table 2.4 Transposons identified in *C. metallidurans* CH34

Transposon	Family	Accessory genes	Function	Length (kb)	CHR	Chromid	pMOL28	pMOL30
Tn*4378*	Tn*21*/Tn*501*	*merRTPADEurf-2*	Mercury resistance	8.2			1	
Tn*4380*	Tn*21*/Tn*501*	*merRTPADEurf-2*	Mercury resistance	8.0				1
Tn*6048*	Tn*5053*	*mmfABC$_1$DC$_2$RSM*	Unknown, induced by Zn and Pb	10.4	1	2		
Tn*6049*		(*gspA*)	Unknown	3.5	7	3	1	1
Tn*6050*	Tn*21*/Tn*501*	*sulP*, *uspA*, *dksA*	Unknown	6.8		2		

interphylum horizontal gene transfer. This is supported by the fact that both pMAB01 and BRA100 are BHR plasmids from the IncP-1β subgroup (Leao et al. 2013). The Tn*4380 mer* genes are also observed with 100 % identity in the IncP-1β plasmid pJP4 from *C. pinatubonensis* JMP134, mainly studied for its herbicide degradation genes (Don and Pemberton 1985; Lykidis et al. 2010), and other BHR plasmids such as the IncP-1β plasmid pADB1 (Martinez et al. 2001) and the IncP-1γ plasmid pQKH54 (Haines et al. 2006).

While Tn*4378* and Tn*4380* carry mercury resistance determinants (see Chap. 3), Tn*6048* and Tn*6050* carry other less common accessory genes. Tn*6048* harbours the *mmfABC$_1$DC$_2$RSM* cluster encoding a two-component regulatory system (MmfRS) putatively regulating the expression of a peptidase (MmfA), a phosphatase (MmfB), two uncharacterized membrane-anchored proteins (MmfC$_1$ and MmfC$_2$) and a drug/metabolite transporter (DMT) super family permease (MmfM). Both MmfC$_1$ and MmfC$_2$ show high similarity (37.9 and 78.3 %, respectively) with ZnfP encoded by the *zni-znf-zne* cluster on the chromid (Sect. 2.4.1.2). Transposons highly similar to Tn*6048* were found in *C. metallidurans* NA1 (>97 % nucleotide similarity), *Burkholderia* sp. TJI49 (>99 % nucleotide similarity) and in plasmid pBVIE02 from *B. vietnamiensis* G4 (>98 % nucleotide similarity; disrupted by IS insertions). In addition, clusters similar to the *mmf* cluster were found in *Acidovorax delafieldii* 2AN and *Dechloromonas aromatica* RCB. Although the function of this *mmf* cluster is still unknown, these genes apparently participate in the response to heavy metals as they are strongly induced in the presence of zinc and lead. Furthermore, MmfB shows 38 % sequence similarity to BcrC of *Bacillus subtilis*, which has undecaprenyl pyrophosphate phosphatase activity (Bernard et al. 2005), and the undecaprenyl pyrophosphate phosphatase activity of PbrB has been shown to be involved in lead resistance (Hynninen et al. 2009; Taghavi et al. 2009). In addition, overexpression of undecaprenyl pyrophosphate phosphatase in *Bacillus subtilis* increased resistance to bacitracin, which prevents cell wall synthesis by inhibiting the dephosphorylation of undecaprenyl pyrophosphate, and the rare earth element scandium (Bernard et al. 2005; Inaoka and Ochi 2012). Noteworthy, identical copies of Tn*6048* were also identified on BHR plasmids pMOL98 (Van der Auwera et al. 2009) and pAKD16 (Sen et al. 2011). However, both plasmids probably captured Tn*6048* as a result of the used exogenous isolation procedure with a CH34 derivative as recipient (Top et al. 1994; Van der Auwera et al. 2009; Sen et al. 2011). The accessory genes of Tn*6050* code for a sulfate permease (SulP), a universal stress protein (UspA) and a DnaK suppressor-like protein (DksA). Their role is still unknown but a functional association could be assumed as these accessory genes (or a part) are found alone or in combination with antibiotic resistance determinants in other Tn*3*-related transposons such as Tn*6001*, Tn*1403* and Tn*1404** derived from clinical and environmental *Pseudomonas* strains (Tseng et al. 2007; Stokes et al. 2007), and Tn*1013* from the IncP-1α plasmid pBS228 (Haines et al. 2007). The DksA protein has been shown to be a global transcriptional regulator that acts as co-regulator along with the archetypical alarmone ppGpp for controlling the stringent response (Paul et al. 2005; Kanjee et al. 2012). Furthermore, the redox active thiols in the 4-cysteine zinc-finger motif of DksA

(which is also present in the DksA encoded by Tn*6050*) sense oxidative and nitrosative stress, thereby fine-tuning the expression of the translational machinery and amino acid assimilation and biosynthesis (Henard et al. 2014). Therefore, although speculative, Tn*6050* could contribute to the adaptation to metabolic stress imposed by harsh environmental conditions.

These observations illustrate the modular character (of this group) of transposons, that is, different transposition modules associated with diverse mercury resistance cassettes as well as other accessory genes.

Transposon Tn*6049* harbours four genes (*tnmB*, *gspA*, *tnmA*, and *tnmC*). The GspA protein sequence shows a conserved domain related to ATPases associated with type II protein secretion systems. However, the function of its putative accessory genes is still unknown. The association of Tn*6049* with genomic islands is striking (at least 8 out of the 12 copies) (Van Houdt et al. 2009). Therefore, it appears to play a peculiar role in "stabilizing" genomic islands. Furthermore, inactivating transposase/integrase genes should immobilize and fix the affected genomic island in the genome.

2.5 Concluding Remarks

The genome of *C. metallidurans* CH34 harbours a high number and diversity of mobile genetic elements such as plasmids, genomic islands, integrative and conjugative elements, transposons and insertion sequence elements, but also novel types of putative elements such as the RIT elements, BIM and PRQ modules. Many of these elements contribute to its adaptation potential to certain niches. In particular for heavy metal resistance, an important role for the megaplasmids pMOL28 and pMOL30 has repeatedly been demonstrated, conferring resistance to cadmium, chromate, cobalt, copper, mercury, nickel, lead and zinc. Since the backbones of these megaplasmids are similar to those found in other strains unable to withstand high heavy metal concentrations, particular traits probably integrated into an ancestor, resulting in pMOL28 and pMOL30. Other mobile genetic elements that contribute to the heavy metal response range from genomic islands and transposons carrying specific accessory genes to insertion sequences that affect regulatory functions.

In addition to these mobile genetic elements, also the CH34 chromid carries a diversified reservoir of metal response genes. For instance, the majority of the RND-driven efflux systems are located on the chromid, although most of these systems are inactivated or have unknown functions. Inactivation of these efflux systems could be due to redundancy by the acquisition of pMOL28 and pMOL30. Alternatively, these efflux systems acquired by an ancestor of CH34 could efflux elements rarely found in high concentrations in modern industrialized environments, thereby relaxing selection pressure.

While most metal resistance determinants are shared by all *C. metallidurans* strains, irrespective of the strain's isolation type and place (bear in mind that all

isolates come from anthropized environments), substantial differences in the diversity and size of their mobile gene pool was observed. For instance, reflected by a strain's ability to degrade toluene or to grow on hydrogen gas and carbon dioxide. The varying genomic and genetic contexts can affect the interaction patterns and phenotypic compensations between the involved proteins, therefore, comparative studies using different *C. metallidurans* strains may be helpful to map the diversity in regulatory circuits and to characterize unknown response mechanisms to stressors and heavy metals in particular. Furthermore, comparative studies will allow revealing the full adaptation potential of the *C. metallidurans* species to heavy metals.

References

Alvarez AF, Rodriguez C, Georgellis D (2013) Ubiquinone and menaquinone electron carriers represent the yin and yang in the redox regulation of the ArcB sensor kinase. J Bacteriol 195 (13):3054–3061

Amadou C, Pascal G, Mangenot S, Glew M, Bontemps C, Capela D, Carrere S, Cruveiller S, Dossat C, Lajus A, Marchetti M, Poinsot V, Rouy Z, Servin B, Saad M, Schenowitz C, Barbe V, Batut J, Medigue C, Masson-Boivin C (2008) Genome sequence of the beta-rhizobium *Cupriavidus taiwanensis* and comparative genomics of rhizobia. Genome Res 18(9):1472–1483

Bentley SD, Corton C, Brown SE, Barron A, Clark L, Doggett J, Harris B, Ormond D, Quail MA, May G, Francis D, Knudson D, Parkhill J, Ishimaru CA (2008) Genome of the actinomycete plant pathogen *Clavibacter michiganensis* subsp. *sepedonicus* suggests recent niche adaptation. J Bacteriol 190(6):2150–2160

Bernard R, El Ghachi M, Mengin-Lecreulx D, Chippaux M, Denizot F (2005) BcrC from *Bacillus subtilis* acts as an undecaprenyl pyrophosphate phosphatase in bacitracin resistance. J Biol Chem 280(32):28852–28857

Bersch B, Derfoufi KM, De Angelis F, Auquier V, Ekende EN, Mergeay M, Ruysschaert JM, Vandenbussche G (2011) Structural and metal binding characterization of the C-terminal metallochaperone domain of membrane fusion protein SilB from *Cupriavidus metallidurans* CH34. Biochemistry 50(12):2194–2204

Bickhart DM, Gogarten JP, Lapierre P, Tisa LS, Normand P, Benson DR (2009) Insertion sequence content reflects genome plasticity in strains of the root nodule actinobacterium *Frankia*. BMC Genom 10:468

Bondarczuk K, Piotrowska-Seget Z (2013) Molecular basis of active copper resistance mechanisms in gram-negative bacteria. Cell Biol Toxicol 29(6):397–405

Borremans B, Hobman JL, Provoost A, Brown NL, van der Lelie D (2001) Cloning and functional analysis of the *pbr* lead resistance determinant of *Ralstonia metallidurans* CH34. J Bacteriol 183(19):5651–5658

Brim H, Heyndrickx M, de Vos P, Wilmotte A, Springael D, Schlegel HG, Mergeay M (1999) Amplified rDNA restriction analysis and further genotypic characterisation of metal-resistant soil bacteria and related facultative hydrogenotrophs. Syst Appl Microbiol 22(2):258–268

Brown NL, Evans LR (1991) Transposition in prokaryotes: transposon Tn*501*. Res Microbiol 142 (6):689–700

Busby JN, Panjikar S, Landsberg MJ, Hurst MR, Lott JS (2013) The BC component of ABC toxins is an RHS-repeat-containing protein encapsulation device. Nature 501(7468):547–550

Chen WJ, Hsieh FC, Hsu FC, Tasy YF, Liu JR, Shih MC (2014) Characterization of an insecticidal toxin and pathogenicity of *Pseudomonas taiwanensis* against insects. PLoS Pathog 10(8):e1004288

Collard JM, Provoost A, Taghavi S, Mergeay M (1993) A new type of *Alcaligenes eutrophus* CH34 zinc resistance generated by mutations affecting regulation of the *cnr* cobalt-nickel resistance system. J Bacteriol 175(3):779–784

Collard JM, Corbisier P, Diels L, Dong Q, Jeanthon C, Mergeay M, Taghavi S, van der Lelie D, Wilmotte A, Wuertz S (1994) Plasmids for heavy metal resistance in *Alcaligenes eutrophus* CH34: mechanisms and applications. FEMS Microbiol Rev 14(4):405–414

Diaz-Perez C, Cervantes C, Campos-Garcia J, Julian-Sanchez A, Riveros-Rosas H (2007) Phylogenetic analysis of the chromate ion transporter (CHR) superfamily. FEBS J 274 (23):6215–6227

Diels L, Mergeay M (1990) DNA probe-mediated detection of resistant bacteria from soils highly polluted by heavy metals. Appl Environ Microbiol 56(5):1485–1491

Diels L, Faelen M, Mergeay M, Nies D (1985) Mercury transposons from plasmids governing multiple resistance to heavy metals in *Alcaligenes eutrophus* CH34. Arch Physiol Biochem 93:27–28

Diels L, Sadouk A, Mergeay M (1989) Large plasmids governing multiple resistances to heavy metals—a genetic approach. Toxicol Environ Chem 23(1–4):79–89

Diels L, Dong QH, van der Lelie D, Baeyens W, Mergeay M (1995) The *czc* operon of *Alcaligenes eutrophus* CH34: from resistance mechanism to the removal of heavy metals. J Ind Microbiol 14(2):142–153

Don RH, Pemberton JM (1981) Properties of six pesticide degradation plasmids isolated from *Alcaligenes paradoxus* and *Alcaligenes eutrophus*. J Bacteriol 145(2):681–686

Don RH, Pemberton JM (1985) Genetic and physical map of the 2,4-dichlorophenoxyacetic acid-degradative plasmid pJP4. J Bacteriol 161(1):466–468

Dong Q, Sadouk A, van der Lelie D, Taghavi S, Ferhat A, Nuyten JM, Borremans B, Mergeay M, Toussaint A (1992) Cloning and sequencing of IS*1086*, an *Alcaligenes eutrophus* insertion element related to IS*30* and IS*4351*. J Bacteriol 174(24):8133–8138

Dubarry N, Pasta F, Lane D (2006) ParABS systems of the four replicons of *Burkholderia cenocepacia*: new chromosome centromeres confer partition specificity. J Bacteriol 188 (4):1489–1496

Fricke WF, Kusian B, Bowien B (2009) The genome organization of *Ralstonia eutropha* strain H16 and related species of the *Burkholderiaceae*. J Microbiol Biotechnol 16(1–2):124–135

Friedrich B, Hogrefe C, Schlegel HG (1981) Naturally occurring genetic transfer of hydrogen-oxidizing ability between strains of *Alcaligenes eutrophus*. J Bacteriol 147 (1):198–205

Frost LS, Leplae R, Summers AO, Toussaint A (2005) Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 3(9):722–732

Grass G, Grosse C, Nies DH (2000) Regulation of the *cnr* cobalt and nickel resistance determinant from *Ralstonia* sp. strain CH34. J Bacteriol 182(5):1390–1398

Gstalder ME, Faelen M, Mine N, Top EM, Mergeay M, Couturier M (2003) Replication functions of new broad host range plasmids isolated from polluted soils. Res Microbiol 154(7):499–509

Haines AS, Akhtar P, Stephens ER, Jones K, Thomas CM, Perkins CD, Williams JR, Day MJ, Fry JC (2006) Plasmids from freshwater environments capable of IncQ retrotransfer are diverse and include pQKH54, a new IncP-1 subgroup archetype. Microbiology 152(Pt 9):2689–2701

Haines AS, Jones K, Batt SM, Kosheleva IA, Thomas CM (2007) Sequence of plasmid pBS228 and reconstruction of the IncP-1alpha phylogeny. Plasmid 58(1):76–83

Harrison PW, Lower RP, Kim NK, Young JP (2010) Introducing the bacterial 'chromid': not a chromosome, not a plasmid. Trends Microbiol 18(4):141–148

Henard CA, Tapscott T, Crawford MA, Husain M, Doulias PT, Porwollik S, Liu L, McClelland M, Ischiropoulos H, Vazquez-Torres A (2014) The 4-cysteine zinc-finger motif of the RNA polymerase regulator DksA serves as a thiol switch for sensing oxidative and nitrosative stress. Mol Microbiol 91(4):790–804

Hong KW, Thinagaran D, Gan HM, Yin WF, Chan KG (2012) Whole-genome sequence of *Cupriavidus* sp. strain BIS7, a heavy-metal-resistant bacterium. J Bacteriol 194(22):6324

Hubner A, Hendrickson W (1997) A fusion promoter created by a new insertion sequence, IS*1490*, activates transcription of 2,4,5-trichlorophenoxyacetic acid catabolic genes in *Burkholderia cepacia* AC1100. J Bacteriol 179(8):2717–2723

Hunter S, Jones P, Mitchell A, Apweiler R, Attwood TK, Bateman A, Bernard T, Binns D, Bork P, Burge S, de Castro E, Coggill P, Corbett M, Das U, Daugherty L, Duquenne L, Finn RD, Fraser M, Gough J, Haft D, Hulo N, Kahn D, Kelly E, Letunic I, Lonsdale D, Lopez R, Madera M, Maslen J, McAnulla C, McDowall J, McMenamin C, Mi H, Mutowo-Muellenet P, Mulder N, Natale D, Orengo C, Pesseat S, Punta M, Quinn AF, Rivoire C, Sangrador-Vegas A, Selengut JD, Sigrist CJ, Scheremetjew M, Tate J, Thimmajanarthanan M, Thomas PD, Wu CH, Yeats C, Yong SY (2012) InterPro in 2011: new developments in the family and domain prediction database. Nucleic Acids Res 40(Database issue): D306–D312

Hynninen A, Touze T, Pitkanen L, Mengin-Lecreulx D, Virta M (2009) An efflux transporter PbrA and a phosphatase PbrB cooperate in a lead-resistance mechanism in bacteria. Mol Microbiol 74(2):384–394

Inaoka T, Ochi K (2012) Undecaprenyl pyrophosphate involvement in susceptibility of *Bacillus subtilis* to rare earth elements. J Bacteriol 194(20):5632–5637

Jackson AP, Thomas GH, Parkhill J, Thomson NR (2009) Evolutionary diversification of an ancient gene family (rhs) through C-terminal displacement. BMC Genom 10:584

Janssen PJ, Van Houdt R, Moors H, Monsieurs P, Morin N, Michaux A, Benotmane MA, Leys N, Vallaeys T, Lapidus A, Monchy S, Médigue C, Taghavi S, McCorkle S, Dunn J, van der Lelie D, Mergeay M (2010) The complete genome sequence of *Cupriavidus metallidurans* strain CH34, a master survivalist in harsh and anthropogenic environments. PLoS ONE 5(5): e10433

Juhnke S, Peitzsch N, Hubener N, Grosse C, Nies DH (2002) New genes involved in chromate resistance in *Ralstonia metallidurans* strain CH34. Arch Microbiol 179(1):15–25

Kanjee U, Ogata K, Houry WA (2012) Direct binding targets of the stringent response alarmone (p)ppGpp. Mol Microbiol 85(6):1029–1043

Kholodii GY, Mindlin SZ, Bass IA, Yurieva OV, Minakhina SV, Nikiforov VG (1995) Four genes, two ends, and a res region are involved in transposition of Tn*5053*: a paradigm for a novel family of transposons carrying either a *mer* operon or an integron. Mol Microbiol 17 (6):1189–1200

Klockgether J, Wurdemann D, Reva O, Wiehlmann L, Tummler B (2007) Diversity of the abundant pKLC102/PAGI-2 family of genomic islands in *Pseudomonas aeruginosa*. J Bacteriol 189(6):2443–2459

Klockgether J, Wurdemann D, Wiehlmann L, Tummler B (2008) Transcript profiling of the *Pseudomonas aeruginosa* genomic islands PAGI-2 and pKLC102. Microbiology 154(Pt 6):1599–1604

Koskiniemi S, Lamoureux JG, Nikolakakis KC, de Roodenbeke CTK, Kaplan MD, Low DA, Hayes CS (2013) Rhs proteins from diverse bacteria mediate intercellular competition. Proc Natl Acad Sci U S A 110(17):7032–7037

Koskiniemi S, Garza-Sanchez F, Sandegren L, Webb JS, Braaten BA, Poole SJ, Andersson DI, Hayes CS, Low DA (2014) Selection of orphan Rhs toxin expression in evolved *Salmonella enterica* serovar Typhimurium. PLoS Genet 10(3):e1004255

Kung VL, Khare S, Stehlik C, Bacon EM, Hughes AJ, Hauser AR (2012) An rhs gene of *Pseudomonas aeruginosa* encodes a virulence protein that activates the inflammasome. Proc Natl Acad Sci U S A 109(4):1275–1280

Larbig KD, Christmann A, Johann A, Klockgether J, Hartsch T, Merkl R, Wiehlmann L, Fritz HJ, Tummler B (2002) Gene islands integrated into tRNA(Gly) genes confer genome diversity on a *Pseudomonas aeruginosa* clone. J Bacteriol 184(23):6665–6680

Leao SC, Matsumoto CK, Carneiro A, Ramos RT, Nogueira CL, Lima JD, Jr., Lima KV, Lopes ML, Schneider H, Azevedo VA, da Costa da Silva A (2013) The detection and

sequencing of a broad-host-range conjugative IncP-1beta plasmid in an epidemic strain of *Mycobacterium abscessus* subsp. *bolletii*. PLoS One 8(4): e60746

Legatzki A, Grass G, Anton A, Rensing C, Nies DH (2003) Interplay of the Czc system and two P-type ATPases in conferring metal resistance to *Ralstonia metallidurans*. J Bacteriol 185 (15):4354–4361

Li LG, Cai L, Zhang T (2013) Genome of *Cupriavidus* sp. HMR-1, a heavy metal-resistant bacterium. Genome Announc 1(1):e00202–e00212

Liesegang H, Lemke K, Siddiqui RA, Schlegel HG (1993) Characterization of the inducible nickel and cobalt resistance determinant *cnr* from pMOL28 of *Alcaligenes eutrophus* CH34. J Bacteriol 175(3):767–778

Lin H, Li TY, Xie MH, Zhang Y (2007) Characterization of the variants, flanking genes, and promoter activity of the *Leifsonia xyli* subsp. *cynodontis* insertion sequence IS*1237*. J Bacteriol 189(8):3217–3227

Lykidis A, Perez-Pantoja D, Ledger T, Mavromatis K, Anderson IJ, Ivanova NN, Hooper SD, Lapidus A, Lucas S, Gonzalez B, Kyrpides NC (2010) The complete multipartite genome sequence of *Cupriavidus necator* JMP134, a versatile pollutant degrader. PLoS ONE 5(3): e9729

Mahillon J, Chandler M (1998) Insertion sequences. Microbiol Mol Biol Rev 62(3):725–774

Martinez B, Tomkins J, Wackett LP, Wing R, Sadowsky MJ (2001) Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. J Bacteriol 183(19):5684–5697

Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60

Mela F, Fritsche K, Boersma H, van Elsas JD, Bartels D, Meyer F, de Boer W, van Veen JA, Leveau JH (2008) Comparative genomics of the pIPO2/pSB102 family of environmental plasmids: sequence, evolution, and ecology of pTer331 isolated from *Collimonas fungivorans* Ter331. FEMS Microbiol Ecol 66(1):45–62

Mergeay M, Nies D, Schlegel HG, Gerits J, Charles P, Van Gijsegem F (1985) *Alcaligenes eutrophus* CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. J Bacteriol 162(1):328–334

Mergeay M, Monchy S, Janssen P, Van Houdt R, Leys N (2009) Megaplasmids in *Cupriavidus* genus and metal resistance. In: Schwartz E (ed) Microbial megaplasmids, vol 11., Microbiology MonographsSpringer, Berlin, pp 209–238

Merlin C, Springael D, Toussaint A (1999) Tn*4371*: a modular structure encoding a phage-like integrase, a *Pseudomonas*-like catabolic pathway, and RP4/Ti-like transfer functions. Plasmid 41(1):40–54

Mijnendonckx K, Provoost A, Monsieurs P, Leys N, Mergeay M, Mahillon J, Van Houdt R (2011) Insertion sequence elements in *Cupriavidus metallidurans* CH34: distribution and role in adaptation. Plasmid 65(3):193–203

Mijnendonckx K, Provoost A, Ott CM, Venkateswaran K, Mahillon J, Leys N, Van Houdt R (2013) Characterization of the survival ability of *Cupriavidus metallidurans* and *Ralstonia pickettii* from space-related environments. Microb Ecol 65(2):347–360

Minakhina S, Kholodii G, Mindlin S, Yurieva O, Nikiforov V (1999) Tn*5053* family transposons are *res* site hunters sensing plasmidal *res* sites occupied by cognate resolvases. Mol Microbiol 33(5):1059–1068

Mindlin S, Petrova M (2013) Mercury resistance transposons. In: Roberts AP, Mullany P (eds) Bacterial integrative mobile genetic elements. Landes Biosciences, Austin, pp 33–52

Mira A, Klasson L, Andersson SG (2002) Microbial genome evolution: sources of variability. Curr Opin Microbiol 5(5):506–512

Miyazaki R, Minoia M, Pradervand N, Sentchilo V, Sulser S, Reinhard F, van der Meer JR (2013) The *clc* element and related genomic islands in *Proteobacteria*. In: Roberts AP, Mullany P (eds) Bacterial integrative mobile genetic elements. Landes Bioscience, Austin, pp 261–272

Monchy S, Benotmane MA, Wattiez R, van Aelst S, Auquier V, Borremans B, Mergeay M, Taghavi S, van der Lelie D, Vallaeys T (2006a) Transcriptomic and proteomic analyses of the

pMOL30-encoded copper resistance in *Cupriavidus metallidurans* strain CH34. Microbiology 152(Pt 6):1765–1776

Monchy S, Vallaeys T, Bossus A, Mergeay M (2006b) Metal transport ATPase genes from *Cupriavidus metallidurans* CH34: a transcriptomic approach. Int J Environ Anal Chem 86 (9):677–692

Monchy S, Benotmane MA, Janssen P, Vallaeys T, Taghavi S, van der Lelie D, Mergeay M (2007) Plasmids pMOL28 and pMOL30 of *Cupriavidus metallidurans* are specialized in the maximal viable response to heavy metals. J Bacteriol 189(20):7417–7425

Monsieurs P, Moors H, Van Houdt R, Janssen PJ, Janssen A, Coninx I, Mergeay M, Leys N (2011) Heavy metal resistance in *Cupriavidus metallidurans* CH34 is governed by an intricate transcriptional network. Biometals 24(6):1133–1151

Monsieurs P, Provoost A, Mijnendonckx K, Leys N, Gaudreau C, Van Houdt R (2013) Genome sequence of *Cupriavidus metallidurans* Strain H1130, isolated from an invasive human infection. Genome Announc 1(6):e01051–e01013

Monsieurs P, Mijnendonckx K, Provoost A, Venkateswaran K, Ott CM, Leys N, Van Houdt R (2014) Genome sequences of *Cupriavidus metallidurans* Strains NA1, NA4, and NE12, isolated from space equipment. Genome Announc 2(4):e00719–e00714

Nies DH (1995) The cobalt, zinc, and cadmium efflux system CzcABC from *Alcaligenes eutrophus* functions as a cation-proton antiporter in *Escherichia coli*. J Bacteriol 177 (10):2707–2712

Nies A, Nies DH, Silver S (1990) Nucleotide sequence and expression of a plasmid-encoded chromate resistance determinant from *Alcaligenes eutrophus*. J Biol Chem 265(10):5648–5653

Nies DH, Koch S, Wachi S, Peitzsch N, Saier MH Jr (1998) CHR, a novel family of prokaryotic proton motive force-driven transporters probably containing chromate/sulfate antiporters. J Bacteriol 180(21):5799–5802

Overmars L, Kerkhoven R, Siezen RJ, Francke C (2013) MGcV: the microbial genomic context viewer for comparative genome analysis. BMC Genom 14:209

Paul BJ, Berkmen MB, Gourse RL (2005) DksA potentiates direct activation of amino acid promoters by ppGpp. Proc Natl Acad Sci USA 102(22):7823–7828

Poehlein A, Kusian B, Friedrich B, Daniel R, Bowien B (2012) Complete genome sequence of the type strain *Cupriavidus necator* N-1. J Bacteriol 193(18):5017

Poole SJ, Diner EJ, Aoki SK, Braaten BA, de Roodenbeke CTK, Low DA, Hayes CS (2011) Identification of functional toxin/immunity genes linked to contact-dependent growth inhibition (CDI) and rearrangement hotspot (Rhs) systems. PLoS Genet 7(8):e1002217

Remenant B, de Cambiaire JC, Cellier G, Jacobs JM, Mangenot S, Barbe V, Lajus A, Vallenet D, Médigue C, Fegan M, Allen C, Prior P (2011) *Ralstonia syzygii*, the blood disease bacterium and some Asian *Ralstonia solanacearum* strains form a single genomic species despite divergent lifestyles. PLoS One 6(9): e24356

Ricker N, Qian H, Fulthorpe RR (2013) Phylogeny and organization of recombinase in trio (RIT) elements. Plasmid 70(2):226–239

Ryan MP, Pembroke JT, Adley CC (2009) Novel Tn*4371*-ICE like element in *Ralstonia pickettii* and genome mining for comparative elements. BMC Microbiol 9:242

Sadouk A, Mergeay M (1993) Chromosome mapping in *Alcaligenes eutrophus* CH34. Mol Gen Genet 240(2):181–187

Salanoubat M, Genin S, Artiguenave F, Gouzy J, Mangenot S, Arlat M, Billault A, Brottier P, Camus JC, Cattolico L, Chandler M, Choisne N, Claudel-Renard C, Cunnac S, Demange N, Gaspin C, Lavie M, Moisan A, Robert C, Saurin W, Schiex T, Siguier P, Thébault P, Whalen M, Wincker P, Levy M, Weissenbach J, Boucher CA (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. Nature 415(6871):497–502

Sato Y, Nishihara H, Yoshida M, Watanabe M, Rondal JD, Concepcion RN, Ohta H (2006) *Cupriavidus pinatubonensis* sp. nov. and *Cupriavidus laharis* sp. nov., novel hydrogen-oxidizing, facultatively chemolithotrophic bacteria isolated from volcanic mudflow deposits from Mt. Pinatubo in the Philippines. Int J Syst Evol Microbiol 56(Pt 5): 973–978

Sawana A, Adeolu M, Gupta RS (2014) Molecular signatures and phylogenomic analysis of the genus *Burkholderia*: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia* gen. nov. harboring environmental species. Front Genet 5:429

Schmidt T, Schlegel HG (1989) Nickel and cobalt resistance of various bacteria isolated from soil and highly polluted domestic and industrial wastes. FEMS Microbiol Lett 62(5):315–328

Schmidt T, Stoppel RD, Schlegel HG (1991) High-level nickel resistance in *Alcaligenes xylosoxydans* 31A and *Alcaligenes eutrophus* KTO2. Appl Environ Microbiol 57(11):3301–3309

Schneider D, Lenski RE (2004) Dynamics of insertion sequence elements during experimental evolution of bacteria. Res Microbiol 155(5):319–327

Schneider D, Faure D, Noirclerc-Savoye M, Barrière AC, Coursange E, Blot M (2000) A broad-host-range plasmid for isolating mobile genetic elements in gram-negative bacteria. Plasmid 44(2):201–207

Schwartz E (2009) Microbial megaplasmids. Microbiology Monographs. Springer, Berlin

Schwartz E, Henne A, Cramm R, Eitinger T, Friedrich B, Gottschalk G (2003) Complete nucleotide sequence of pHG1: a *Ralstonia eutropha* H16 megaplasmid encoding key enzymes of H(2)-based ithoautotrophy and anaerobiosis. J Mol Biol 332(2):369–383

Sen D, Van der Auwera GA, Rogers LM, Thomas CM, Brown CJ, Top EM (2011) Broad-host-range plasmids from agricultural soils have IncP-1 backbones with diverse accessory genes. Appl Environ Microbiol 77(22):7975–7983

Sharma P, Stagge S, Bekker M, Bettenbrock K, Hellingwerf KJ (2013) Kinase activity of ArcB from *Escherichia coli* Is subject to regulation by both ubiquinone and demethylmenaquinone. PLoS ONE 8(10):e75412

Siguier P, Gourbeyre E, Chandler M (2014) Bacterial insertion sequences: their genomic impact and diversity. FEMS Microbiol Rev 38(5): 865–891

Silver S, Phung LT (1996) Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol 50:753–789

Sisto A, Cipriani MG, Morea M, Lonigro SL, Valerio F, Lavermicocca P (2010) An Rhs-like genetic element is involved in bacteriocin production by *Pseudomonas savastanoi* pv. *savastanoi*. Anton Leeuw Int J G 98(4):505–517

Stokes HW, Elbourne LD, Hall RM (2007) Tn1403, a multiple-antibiotic resistance transposon made up of three distinct transposons. Antimicrob Agents Chemother 51(5):1827–1829

Taghavi S, Lesaulnier C, Monchy S, Wattiez R, Mergeay M, van der Lelie D (2009) Lead(II) resistance in *Cupriavidus metallidurans* CH34: interplay between plasmid and chromosomally-located functions. Anton Leeuw Int J G 96(2):171–182

Talat ME (2000) Genetic mechanism of heavy metal resistance of *Pseudomonas aeruginosa* CMG103. PhD thesis, University of Karachi, Karachi

Tauch A, Schneiker S, Selbitschka W, Puhler A, van Overbeek LS, Smalla K, Thomas CM, Bailey MJ, Forney LJ, Weightman A, Ceglowski P, Pembroke T, Tietze E, Schroder G, Lanka E, van Elsas JD (2002) The complete nucleotide sequence and environmental distribution of the cryptic, conjugative, broad-host-range plasmid pIPO2 isolated from bacteria of the wheat rhizosphere. Microbiology 148(Pt 6):1637–1653

Tibazarwa C, Wuertz S, Mergeay M, Wyns L, van der Lelie D (2000) Regulation of the *cnr* cobalt and nickel resistance determinant of *Ralstonia eutropha* (*Alcaligenes eutrophus*) CH34. J Bacteriol 182(5):1399–1409

Timotius K, Schlegel HG (1987) Aus Abwässern isolierte nickel-resistente Bakterien. Nachrichten der Akademie der Wissenschaften Göttingen II Math-Physik Klasse 3:15–23

Top E, Desmet I, Verstraete W, Dijkmans R, Mergeay M (1994) Exogenous isolation of mobilizing plasmids from polluted soils and sludges. Appl Environ Microbiol 60(3):831–839

Touchon M, Rocha EP (2007) Causes of insertion sequences abundance in prokaryotic genomes. Mol Biol Evol 24(4):969–981

Toussaint A, Merlin C, Monchy S, Benotmane MA, Leplae R, Mergeay M, Springael D (2003) The biphenyl- and 4-chlorobiphenyl-catabolic transposon Tn*4371*, a member of a new family of genomic islands related to IncP and Ti plasmids. Appl Environ Microbiol 69(8):4837–4845

Tseng SP, Hsueh PR, Tsai JC, Teng LJ (2007) Tn*6001*, a transposon-like element containing the bla_{VIM-3}-harboring integron In450. Antimicrob Agents Chemother 51(11):4187–4190

Van der Auwera GA, Krol JE, Suzuki H, Foster B, Van Houdt R, Brown CJ, Mergeay M, Top EM (2009) Plasmids captured in *C. metallidurans* CH34: defining the PromA family of broad-host-range plasmids. Anton Leeuw Int J G 96(2): 193–204

van Elsas JD, Gardener BB, Wolters AC, Smit E (1998) Isolation, characterization, and transfer of cryptic gene-mobilizing plasmids in the wheat rhizosphere. Appl Environ Microbiol 64 (3):880–889

Van Houdt R, Mergeay M (2012) Plasmids as secondary chromosomes. In: Bell E, Bond J, Klinman J, Masters B, Wells R (eds) Molecular life sciences: an encyclopedic reference: springerreference (www.springerreference.com)

Van Houdt R, Monchy S, Leys N, Mergeay M (2009) New mobile genetic elements in *Cupriavidus metallidurans* CH34, their possible roles and occurrence in other bacteria. Anton Leeuw Int J G 96:205–226

Van Houdt R, Leplae R, Lima-Mendez G, Mergeay M, Toussaint A (2012a) Towards a more accurate annotation of tyrosine-based site-specific recombinases in bacterial genomes. Mob DNA 3(1):6

Van Houdt R, Monsieurs P, Mijnendonckx K, Provoost A, Janssen A, Mergeay M, Leys N (2012b) Variation in genomic islands contribute to genome plasticity in *Cupriavidus metallidurans*. BMC Genom 13:111

Van Houdt R, Toussaint A, Ryan MP, Pembroke JT, Mergeay M, Adley CC (2013) The Tn*4371* ICE family of bacterial mobile genetic elements. In: Roberts AP, Mullany P (eds) Bacterial integrative mobile genetic elements. Landes Bioscience, Austin, pp 179–200

Wagner A, Lewis C, Bichsel M (2007) A survey of bacterial insertion sequences using IScan. Nucleic Acids Res 35(16):5284–5293

Yang F, Pecina DA, Kelly SD, Kim SH, Kemner KM, Long DT, Marsh TL (2010) Biosequestration via cooperative binding of copper by *Ralstonia pickettii*. Environ Technol 31(8–9):1045–1060

Youderian P, Hartzell PL (2007) Triple mutants uncover three new genes required for social motility in *Myxococcus xanthus*. Genetics 177(1):557–566

Chapter 3
Response of *Cupriavidus metallidurans* CH34 to Metals

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Abstract *Cupriavidus metallidurans* CH34 displays resistance to a plethora of metals. Its response and underlying genetic determinants are dissected and detailed metal by metal (from arsenic to zinc). An important role for its megaplasmids pMOL28 and pMOL30 is shown, with high level resistance to cadmium, chromate, cobalt, copper, mercury, nickel, lead and zinc mediated by well-known genes for detoxification that are often accompanied by other functions linked to acute or chronic stress. Nevertheless, metal resistance determinants are also found on the chromid (e.g. to chromate, copper and zinc) as well as on a large genomic island integrated in the chromosome (e.g. to cadmium, lead and mercury). Even the core genome participates in certain responses such as to gold or selenium. Next, we summarized the environmental applications, which were developed based on the knowledge gained by studying these different determinants, and in particular biosensors and soil and water bioremediation. Finally, the general transcriptional response of *C. metallidurans* to sixteen different metals supplied at different concentrations (including acute stress) is discussed within the framework of its intricate regulatory network.

Keywords *Cupriavidus metallidurans* · Metal resistance · Genetic determinants · Transcriptional regulation · Biotechnology

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3.1 From a to Z: Arsenic to Zinc

The response of *Cupriavidus metallidurans* to a variety of metals has been examined in different ways and from various perspectives that do not necessarily overlap: such as the association of resistance with mobile genetic elements (MGEs), the possible applications to environmental bioremediation, and gene and protein expression levels. The following section describes, metal by metal, the response of *C. metallidurans* to each of them, which helps to put in perspective interesting biological features. However, it should not be forgotten that various gene clusters are addressing responses to more than one metal (e.g. the *cnr* genes respond to cobalt and nickel and the *czc* genes to zinc, cadmium and cobalt). Rules that may govern microbial cation homeostasis from outside the bacterial cell have been formerly provided and shape the physiological and functional landscape of the present description (Nies 2007).

3.1.1 Arsenic: A Chromosome-Bound Gene Cluster

Eight genes (Rmet_0327-Rmet_0334) are putatively involved in resistance to arsenic. This *arsERIC$_2$BC$_1$HP* cluster appears to be only conserved in *C. metallidurans* and codes for the S-adenosyl-methionine (SAM)-dependent methyltransferase ArsE, the As^{3+}-responsive transcriptional regulator ArsR (Zhang et al. 2009), the lactoylglutathione lyase (glyoxalase family) protein ArsI, the glutaredoxin-coupled arsenate reductase $ArsC_1$ and thioredoxin-coupled arsenate reductase $ArsC_2$, the arsenite efflux pump ArsB, the NADPH-dependent FMN reductase ArsH, and the Major Facilitator Superfamily (MFS) permease ArsP. Several individual *ars* gene homologues are found on the chromosome and the chromid such as a putative arsenate reductase (Rmet_1421), a putative arsenite efflux pump (Rmet_3991) and five *arsR*-like regulators (see Sect. 3.3.1.1). However, their participation in arsenic resistance has not been studied (Zhang et al. 2009). The complete *arsERIC$_2$BC$_1$HP* cluster is induced by As^{3+} and As^{5+}, and to a lesser extent by Pb^{2+}, Zn^{2+}, Se^{4+}, Co^{2+} and Cd^{2+} (Monsieurs et al. 2011). Dissociation of the ArsR-promoter/operator region complex was observed in vitro for artificially high concentrations of As^{3+}, Bi^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+} (1 mM), and Cd^{2+}, Pb^{2+} and Zn^{2+} (10 mM) (Zhang et al. 2009) (see Sect. 3.3.1.1). The SAM-dependent methyltransferase ArsE neither contains the characteristics nor the conserved amino acid residues of bacterial and eukaryotic As^{3+} SAM-methyltransferases (Ajees et al. 2012) that can detoxify via volatile arsines (Qin et al. 2006; Yuan et al. 2008). Thus, it appears that *C. metallidurans* CH34 mainly processes toxic levels of As^{3+} and As^{5+}, via initial reduction to As^{3+}, by efflux.

3.1.2 Cadmium, A Combination of Various Efflux Systems

Cadmium was one of the first metals for which plasmid-borne resistance was found in *C. metallidurans* CH34 (Mergeay et al. 1978b). Resistance was associated with the megaplasmid pMOL30, which increased the minimal inhibitory concentration (MIC) of $CdCl_2$ fourfold compared to the plasmid-less derivative AE104 (Mergeay et al. 1985). The resistance genes are located in a 25-gene cluster, part of the genomic island CMGI-30a, that is highly conserved in all *C. metallidurans* strains (Van Houdt et al. 2012) and other strains like *R. pickettii* 12J (see Chap. 2). This cluster encodes three different efflux mechanisms comprising the cation diffusion facilitator (CDF) CzcD (Nies 1992) and the P_{IB4}-type ATPase CzcP (Scherer and Nies 2009), which transport ions from the cytoplasm to the periplasm, and the HME-RND (heavy metal Resistance-Nodulation-Division)-driven system CzcCBA, which transports Cd^{2+} from the periplasm to the outside of the cell. Deletion of *czcB* or *czcA* resulted in the complete loss of efflux and in Cd^{2+}, Co^{2+} and Zn^{2+} sensitivity (Nies et al. 1987, 1989b; Nies 1995; Nies and Silver 1989). In contrast, deletion of the *czcC* gene resulted in a decreased cadmium and cobalt efflux and corresponding sensitive phenotype, while zinc efflux and resistance were unaffected. Next to these efflux systems, the gene cluster codes for the two-component regulatory system CzcRS, the regulatory protein CzcI (Diels et al. 1995a; Grosse et al. 1999), the periplasmic protein CzcE (Zoropogui et al. 2008; Grosse et al. 2004) (see volume II), the membrane-bound isoprenylcysteine carboxyl methyltransferase CzcN (Grosse et al. 1999), the MgtC-like ATPase CzcM and the CopQ-like protein CzcJ (see Sect. 3.1.5.5). These pMOL30-located determinants contribute to cadmium resistance together with the P_{IB2}-type ATPases ZntA (chromid), CadA (genomic island CMGI-1 on the chromosome) and PbrA (pMOL30), the chromosomally-encoded CDF proteins DmeF and FieF, and an uncharacterized pMOL30 gene product, thereby increasing the MIC of Cd^{2+} from 82 nM to 236 μM (Scherer and Nies 2009; Monchy et al. 2006b; Legatzki et al. 2003b). Unlike zinc resistance, the four $P_{IB2/4}$-type ATPases contribute more to cadmium resistance than CzcA. While CadA contributes the most, CzcP is not able to mediate much resistance when being the sole ATPase (Scherer and Nies 2009). However, CzcP has a much higher transport rate compared to the three P_{IB2}-type ATPases. Concerning the three CDF proteins, DmeF and FieF contribute little to cadmium resistance, while the contribution of CzcD is similar to that of CzcP, not being effective alone but enhancing the detoxification mediated by other systems (Scherer and Nies 2009). Therefore, both CzcP and CzcD could function as a rapid cadmium (and zinc) exporter before these metals are able to exert their toxic impact (Scherer and Nies 2009; Nies 2013). Finally, the difference in cadmium resistance between deletion of the above-mentioned determinants in AE128 (carrying only pMOL30) and AE104 (plasmid-less) points towards the involvement of an additional and yet uncharacterized pMOL30 gene product (Scherer and Nies 2009).

A higher MIC of cadmium was observed for *C. metallidurans* CH34 and NE12 compared to NA1, NA2 and NA4 (Mijnendonckx et al. 2013). Interestingly, the

latter three do not contain the *pbrR*$_2$ *cadA pbrC*$_2$ cluster located on the genomic island CMGI-1 (Van Houdt et al. 2012), which is consistent with the important role of CadA. In addition, in contrast to CH34, (at least) NA1, NA4 and NE12 carry a functional chromosomally-encoded HME-RND system ($CzcC_2B_2A_2$) that is highly similar to the pMOL30-encoded CzcCBA (see Chap. 2), which is again consistent with a lesser role for CzcA (or CzcA-like proteins) in cadmium resistance. However, varying global genomic and genetic contexts can affect the interaction patterns and phenotypic compensations between the involved proteins, which can be further studied using these or other *C. metallidurans* strains (see Chap. 2).

Finally, studying the effect of cadmium on *C. metallidurans* has also revealed unexpected properties of its staphyloferrin-B siderophore (formerly alcaligin E) encoded by the chromosomal Rmet_1109-Rmet_1118 genes (Gilis et al. 1996; Munzinger et al. 1999). Gilis et al. (1998) observed that the growth of a staphyloferrin B-deficient (*aleB*) CH34 derivative in the presence of cadmium was markedly stimulated by addition of staphyloferrin-B. Furthermore, staphyloferrin-B was shown to interact with cadmium, thereby decreasing its bioavailability and toxicity. The latter suggests that staphyloferrin-B, besides its function in supplying iron to the cell, may provide protection against heavy metal toxicity (Gilis et al. 1996, 1998; also reviewed in Schalk et al. 2011).

3.1.3 Chromate: More Genes Than Previously Expected

Genes involved in chromate resistance are located on plasmid pMOL28 (more precisely CMGI-28a, see Chap. 2) and on the chromid. The *chrIA*$_1$*B*$_1$*CEF*$_1$ (Rmet_6204-Rmet_6199) cluster on pMOL28 codes for the regulatory proteins ChrI and $ChrF_1$, the chromate efflux pump $ChrA_1$, the putative transcriptional activator $ChrB_2$, the Fe-containing superoxide dismutase-like protein ChrC, which appears to have limited activity (Roux and Coves 2002; Juhnke et al. 2002), and ChrE with sequence similarity to members of the rhodanese superfamily (cleavage of chromium-glutathione complexes) (Juhnke et al. 2002). The cluster on the chromid also codes for the chromate efflux pump $ChrA_2$ (Rmet_3865), the transcriptional repressor $ChrF_2$ (Rmet_3864) and the putative transcriptional activator $ChrB_2$ (Rmet_3866) (Juhnke et al. 2002). Both $ChrA_1$ and $ChrA_2$ belong to the CHR2 subgroup (Diaz-Perez et al. 2007) of the CHR transporter family (first described by Nies et al. 1998).

It became apparent that the pMOL28 cluster contains an additional five genes. The extension of the pMOL28 chromate cluster was based on their strong induction by chromate (Monsieurs et al. 2011), their synteny in the genomes of other bacteria including *Burkholderia pseudomallei*, *Methylobacterium* sp. 4-46 and *Arthrobacter* sp. FB24, and the proteomic response and qRT-PCR analysis of *Arthrobacter* sp. FB24 to chromate (Henne et al. 2009a, b). Accordingly, these genes immediately downstream of the chromate-regulated operon (Rmet_6199 through Rmet_6204) were named *chrO* (Rmet_6198), *chrN* (Rmet_6197), *chrP* (Rmet_6196, encoding a

MFS permease with 10 transmembrane α-helical segments), *chrL* (Rmet_6195, encoding a putative membrane protein), and *chrK* (Rmet_6194, with a WD40/YVTN repeat-like-containing domain) (Henne et al. 2009a).

As a structural analogue of sulphate, chromate enters the cell through sulphate uptake systems (Ohtake et al. 1987) and decreased uptake because of repressed sulphate uptake systems largely contributes to chromate resistance (Juhnke et al. 2002; Nies et al. 1989a). The induction by chromate of genes involved in sulphate and sulphite metabolism (*cysGNDHUIVB*; Rmet_2811-Rmet_2815), in cysteine biosynthesis and (thio)sulphate transport (*cysTWAB*; Rmet_1376-Rmet_1379), and in the formation of the thio-ester binding in acetyl coenzyme A synthesis (Rmet_0607-Rmet_0611) also illustrated the tight relationship between the chromate response and the sulphur metabolism (Monsieurs et al. 2011).

3.1.4 Cobalt: A Substrate of Zinc and Nickel Efflux Systems

Resistance to cobalt was already detected during the early characterization of CH34 (Mergeay et al. 1978a, 1985) and was attributed together with nickel resistance to the *cnr* determinant on pMOL28 (Grass et al. 2000; Liesegang et al. 1993; Siddiqui et al. 1989; Tibazarwa et al. 2000) and together with cadmium and zinc to the *czc* determinant on pMOL30 (Nies et al. 1987; Nies and Silver 1989; Monchy et al. 2007). Resistance to cobalt is also mediated by the *ncc* determinant in other strains such as *C. metallidurans* 31A [carried by plasmid pTOM8 and pTOM9 (Schmidt and Schlegel 1994; Schmidt et al. 1991)], *C. metallidurans* KT02 and strains isolated from New-Caledonia (Schmidt et al. 1991; Stoppel and Schlegel 1995). These genes are very similar to their *cnr* counterpart (see Sect. 3.1.9) but confer a much higher level of resistance to nickel and cobalt. The cluster also contains *nccN*, orthologous to *czcN*, which codes for a putative protein-S-isoprenylcysteine O-methyltransferase possibly involved in C-terminal protein amino acid methylation.

Both HME-RND-driven efflux systems (CzcCBA and CnrCBA) depend on the CDF protein DmeF, which is the most important factor for cobalt resistance (Scherer and Nies 2009; Nies 2013; Munkelt et al. 2004). In contrast to the three P_{IB2}-type ATPases (ZntA, CadA, PbrA), the P_{IB4}-type ATPase CzcP is able to transport cobalt. The contribution of CzcP is similar to that of the CDF protein CzcD, not being effective alone but enhancing the detoxification mediated by other systems (Scherer and Nies 2009; Nies 2013).

In addition, deletion of *atmA* (Rmet_0391) coding for an ATP-binding cassette transporter decreased resistance to cobalt and nickel. AtmA is homologous to Atm1p in the yeast *Saccharomyces cerevisiae*, which is involved in the transport of iron-sulphur cluster-containing proteins. Since AtmA does not transport cobalt or nickel, it is likely to be involved in the transport of compounds required to repair the damage done by periplasmic cobalt or nickel ions (Mikolay and Nies 2009).

3.1.5 Copper: A Large Gene Cluster on pMOL30

3.1.5.1 The *C. metallidurans* CH34 Genomic Landscape of Copper Resistance and Homeostasis Genes

Plasmid-encoded copper resistance was rapidly detected in *C. metallidurans* CH34 (Mergeay et al. 1978b). However, the difference in the MIC between the parental strain and its plasmid-less derivative AE104 is only a factor of 2 to 3, despite the large number of pMOL30 genes that are induced by copper (Monchy et al. 2007, 2006a). However, genome sequencing and additional physiological studies have shown that counterparts of these pMOL30 genes are also present on the chromosome and on the chromid.

3.1.5.2 The Structure of the pMOL30 *cop* Resistance Gene Cluster

The pMOL30 genomic island CMGI-30b contains a 33-gene region that is almost completely induced by Cu^{2+} (Fig. 3.1; Tables 3.1 and 3.2) (Monchy et al. 2006a, 2007; Monsieurs et al. 2011; Mergeay et al. 2009). Within this region, the *cop* gene cluster is unique in respect to its complexity compared to other copper resistance mechanisms described so far. The 21 *cop* genes (Table 3.1), starting with *copV* (Rmet_6105) and ending with *copW* (Rmet_6124), are flanked by two partially deleted genes coding for tyrosine-based site-specific recombinases (Rmet_6103 and *resU*) that may have joined two small genomic islands with components identifiable in other MGEs (Van Houdt et al. 2009, 2013; Ryan et al. 2009). Next to *resU* (Rmet_6125), four copper-induced genes are apparently not directly involved in copper transport or processing but in membrane-related functions with $gtrM_2$ (Rmet_6130) and $gtrB_2$ (Rmet_6128) encoding for a glycosyl transferase, $gtrA_2$ (Rmet_6129) for a bactoprenol-linked glucose translocase, and $ompP_2$ (Rmet_6127) for a porin predicted to form a channel for the diffusion of small hydrophilic molecules (Table 3.2). Orthologs of these four genes, which are located on the genomic island CMGI-30a carrying the *czc* resistance genes (see Chap. 2), are also 100 % conserved in *R. pickettii* 12J as well as in other *C. metallidurans* strains. It could be hypothesized that the *gtrMAB* genes are involved in the

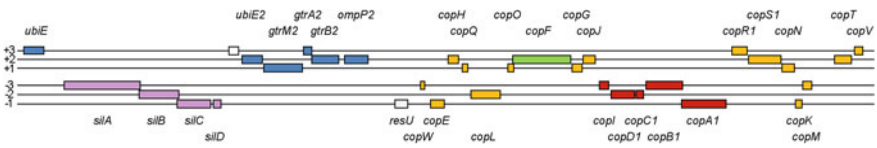

Fig. 3.1 Genomic context of the *cop* cluster on pMOL30 of *C. metallidurans* CH34. Genes coding for the efflux P_I-type ATPase CopF (*green*), the HME-RND-driven efflux system SilCBA (*purple*), the periplasmic detoxification system CopABCDI (*red*), the accessory proteins (*yellow*) and membrane-related functions (*blue*) are shown

Table 3.1 Characteristics of the proteins encoded by the *cop* gene cluster of *C. metallidurans* CH34

Locus tag	Name	AA[a]	Putative function	Localization
Rmet_6105	*copV*	117	Unknown	Cytoplasm
Rmet_6106	*copT*	254[b]	Cytochrome oxidase	Periplasm
Rmet_6107	*copM*	136[b]	Unknown	Periplasm
Rmet_6108	*copK*	94[b]	Effective sequestration Cu^{+} and Cu^{2+}	Periplasm
Rmet_6109	*copN*	164	Unknown	Cytoplasm
Rmet_6110	$copS_1$	463[b]	Histidine kinase	Inner membrane
Rmet_6111	$copR_1$	228	Response regulator	Cytoplasm
Rmet_6112	$copA_1$	614[b]	Multicopper oxidase	Periplasm
Rmet_6113	$copB_1$	495[b]	Cu^{+} storage	Outer membrane
Rmet_6114	$copC_1$	132[b]	Interacts with CopD transporter	Periplasm
Rmet_6115	$copD_1$	305[b]	Transporter (import)	Inner membrane
Rmet_6116	*copI*	158[b]	Copper oxidase	Periplasm
Rmet_6117	*copJ*	174	Participation to the metallic thiol pool	Cytoplasm
Rmet_6118	*copG*	137	Participation to the metallic thiol pool	Cytoplasm
Rmet_6119	*copF*	805[b]	Copper efflux P-ATPase	Inner membrane
Rmet_6382	*copO*	63	Chaperone of CopF	Cytoplasm
Rmet_6120	*copL*	421	Participation to the metallic thiol pool	Cytoplasm
Rmet_6121	*copQ*	78[b]	Highly induced by heavy metals, part of a family of small proteins	Periplasm
Rmet_6122	*copH*	153[b]	Putatively involved in a late stage of the copper response	Periplasm
Rmet_6123	*copE*	211	Unknown but required for maximal expression of copper resistance	Cytoplasm
Rmet_6124	*copW*	69[b]	Unknown, related to CopQ	Periplasm

[a]AA: protein size (number of amino acids)
[b]Size of unprocessed protein, i.e. signal peptide containing form

membrane repair process elicited by metallic stress and that the $OmpP_2$ porin assists the transport of extruded metals to the exterior of the cell. As far as $gtrM_2$ and $gtrB_2$ are concerned, we note that an *Acidiphilium* gene also encoding a glycosyl transferase conferred increased nickel resistance when transferred to an *Escherichia coli* recipient (San Martin-Uriz et al. 2014). This observation suggested the possible relevance of these functions for metal resistance phenotypes. The *silDCBA* (Rmet_6133-6136) cluster, which is mainly induced by Ag^{+} but also by Cu^{2+} (at least *silA* and *silC*), and *ubiE* (Rmet_6137) complete the region of interest for the resistance/response to toxic amounts of copper (Fig. 3.1; Table 3.2).

Table 3.2 Characteristics of the proteins encoded by the copper-induced genes adjacent to the pMOL30 *cop* genes of *C. metallidurans* CH34

Locus tag	Name	AA[a]	Putative function	Localization
Rmet_6127	$ompP_2$	355[b]	Porin predicted to form a channel for the diffusion of small hydrophilic molecules	Outer membrane
Rmet_6128[c]	$gtrB_2$	367	Glycosyltransferase	Inner membrane
Rmet_6129	$gtrA_2$	127	Bactoprenol-linked glucose translocase	Inner membrane
Rmet_6130	$gtrM_2$	541	Glycosyltransferase	Inner membrane
Rmet_6131	$ubiE_2$	289	Methyltransferase	Cytoplasm
Rmet_6132		131	Conserved hypothetical protein	Unknown
Rmet_6133	*silD*	131	Conserved hypothetical protein	Inner membrane
Rmet_6134	*silC*	435[b]	Outer membrane protein of the SilCBA HME-RND efflux system	Outer membrane
Rmet_6135	*silB*	521[b]	Membrane fusion protein of the SilCBA HME-RND efflux system	Periplasm
Rmet_6136	*silA*	1056[b]	Efflux pump of the SilCBA HME-RND efflux system	Inner membrane
Rmet_6137	*ubiE*	290	Methyltransferase	Cytoplasm

[a]AA: protein size (number of amino acids)
[b]Size of unprocessed protein, i.e. signal peptide containing form
[c]Not induced by Cu^{2+}

3.1.5.3 The Three Major Copper Detoxification Systems

The whole pMOL30 copper-induced region codes for three well described copper detoxification systems. The P_{IB1}-type ATPase CopF belongs to the copper efflux subdivision of the P-ATPases (Monchy et al. 2006b; Mergeay et al. 2003) involved in cytoplasmic detoxification. It is assisted by the HME-RND-driven efflux system SilDCBA (Cus-like) (see Sect. 3.1.11) and by the periplasmic copper detoxification system CopABCD. This combination of three different mechanisms of copper resistance is quite frequently observed in a variety of bacteria and linked to mobile genetic elements (Hobman and Crossman 2015). Each system has an additional non-plasmidic counterpart involved in copper detoxification: CupA (*cupRAC;* Rmet_3523-Rmet_3525; chromosome) codes for a P_{IB1}-type ATPase (Wiesemann et al. 2013), *cusDCBAF* (Rmet_5030-Rmet_5034; chromid) encodes a HME-RND-driven efflux system (Auquier 2006; Nies et al. 2006) and $copS_2R_2A_2B_2C_2D_2$ (Rmet_5673-Rmet_5668; chromid) is involved in periplasmic detoxification (Monsieurs et al. 2011).

In order to avoid any confusion over gene or phenotypic designations, Table 3.3 summarizes the closest homologues of the three *C. metallidurans* CH34

Table 3.3 Homologues of *C. metallidurans* CH34 copper resistance proteins

Function	*C. metallidurans*	*E. coli*	*P. syringae*	*E. hirae*
Periplasmic detoxification	$CopA_1B_1C_1D_1$/ $CopA_2B_2C_2D_2$	PcoABCD	CopABCD	
Multicopper oxidase	$CopA_1$/$CopA_2$	PcoA (56 %/56 %) CueO (40 %/38 %)	CopA (55 %/56 %)	
Efflux P_{IB1}-type ATPase	CupA/CopF	CopA (41 %/44 %)		CopA (46 %/41 %) CopB (56 %/57 %)
HME-RND-driven efflux	CusCBA/SilCBA	CusCBA (65 %/66 %)[a]		

The protein sequence similarity is given in parentheses, [a]based on efflux pump (A protein)

detoxification systems in *E. coli*, *Pseudomonas syringae* or *Enterococcus hirae* (i.e. bacteria in which copper homeostasis was extensively studied).

The *C. metallidurans copABCD* genes are homologous to the equivalent *E. coli pco* and *P. syringae cop* genes. The *E. coli pcoABCDRSE* resistance is regulated by the two-component regulatory system PcoRS and contains an additional gene, *pcoE*, encoding a periplasmic copper sponge protein (Zimmermann et al. 2012). The mechanism for *pco* copper resistance is not fully elucidated. Phenotypically, *pco* carrying *E. coli* strains change colour to a dark green/khaki in the presence of excess copper, and there is believed to be a role for pigments/catechol-siderophores in deposition of copper pigments in the cell. The current model for *pco* resistance is that PcoA oxidizes Cu^+ bound to PcoC in the periplasm to less toxic Cu^{2+}. PcoB is predicted to be an outer membrane protein, which is thought to interact with PcoA/C to export copper or to sequestrate oxidized catechol siderophores (Djoko et al. 2008; Rensing and Grass 2003). The role of PcoD is believed to be either to import copper into the cytoplasm, or that PcoC chaperones copper to PcoD which loads it on to periplasmic PcoA which exports it to the periplasm via the twin-arginine translocation pathway (Djoko et al. 2008; Rensing and Grass 2003). Some bacteria may only have the *pcoAB* or *pcoCD* genes. For instance, *Caulobacter crescentus* only uses PcoAB to detoxify copper and deletion results in a 50-fold increased sensitivity (Emeline Lawarée and Jean-Yves Matroule, pers. comm.). There are increasing reports of *pco* and *sil* genes being found together within genomic islands, integrative and conjugative elements, and plasmids [e.g. on the IncH plasmid R478, originally isolated from *Serratia marcescens* (Gilmour et al. 2004), in the chromosome of enterotoxigenic *E. coli* strain H10407 (Crossman et al. 2010) and in *Klebsiella pneumoniae* multiresistance plasmid pUUH239.2 (Sandegren et al. 2012)] (reviewed in Hobman and Crossman 2015).

As mentioned above, the pMOL30-encoded *copSRABCD* genes have counterparts on the chromid, however, there is a major difference in protein sequence between the pMOL30- and chromid-encoded outer membrane protein CopB. The

Fig. 3.2 The CopB motif generated via WebLogo (Crooks et al. 2004) using 434 hits in 119 UniProtKB/TrEMBL sequences. The height of the motif logo represents the bits score, which corresponds to the degree of conservation

pMOL30-encoded CopB contains a 22-fold repeated motif (Fig. 3.2) totalling 44 methionine residues (while the chromid-encoded one only contains 6 methionine residues). This basic motif is observed in other CopB proteins[1] although the number of repeated motifs varies (Fig. 3.3). The whole region ends with a M-[KQ]-M-[KQ] motif and is delimited by (G)-G-S residues especially in the *Cupriavidus* and *Ralstonia* genera. Since it is known that methionine residues are important in binding monovalent copper ions, the observed difference between both CopB proteins may indicate a specialization of the pMOL30-encoded one in the detoxification or neutralization of very high Cu^{+} concentrations.

3.1.5.4 CopK and CopH

Next to the well-identifiable and documented orthologues of *copSRABCD* and *copF*, most of the other *cop* genes, which were first detected in strain CH34 and later in other *C. metallidurans* genomes, remain to be fully deciphered (Table 3.1). They are often absent in most of the conserved synteny blocks involving *cop* genes (and especially those encoding for the three major mechanisms of copper detoxification). It may be hypothesized that such accessory genes are mainly involved in chronic and intense copper stress. Molecular data are currently only available for CopK and CopH.

CopK has been the most intensively studied up to now (Sarret et al. 2010; Chong et al. 2009; Bersch et al. 2008; Monchy et al. 2006a; Tricot et al. 2005). This periplasmic protein, which is one of the most abundant proteins in the periplasmic space during copper challenge (Ruddy Wattiez, pers. comm.), contains seven

[1]ScanProsite (de Castro et al. 2006) detected 119 protein sequences with at least 2 hits for the {M-Q-G-M-D} motif on all UniProtKB/TrEMBL (release 2014_05 of 14-May-2014: 56010222 entries) database sequences.

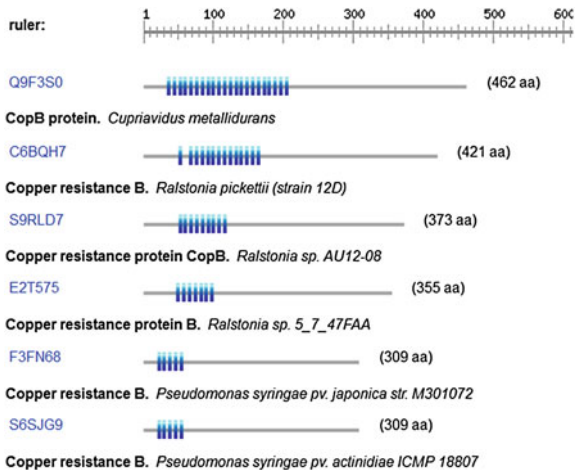

Fig. 3.3 Some examples of CopB proteins with a varying number of motifs (*blue bar*)

methionine residues, five of which appear to be highly conserved, and a tetrathioether-Cu^{+} site (Sarret et al. 2010; Bersch et al. 2008). It is the first protein for which a cooperative binding of Cu^{+} and Cu^{2+} has been observed (Ash et al. 2011; Chong et al. 2009). Binding of Cu^{2+} increased the Cu^{+} affinity of CopK by a factor of 100 and binding of Cu^{+} increased the Cu^{2+} affinity by a factor of at least 10^{6} (Chong et al. 2009). It could be hypothesized that in the presence of high copper levels, CopK strongly chelates the periplasmic copper fraction that could not be timely processed by the ATPase and the periplasmic detoxification system. Therefore, CopK could be seen as an additional copper detoxification system in *C. metallidurans* CH34, especially during chronic and intense metal stress where its sequestration power could prevent saturation of the other systems.

CopH is a paralogue of CzcE, which is involved in the regulation of the *czc* gene cluster (Petit-Haertlein et al. 2010; von Rozycki and Nies 2009; Zoropogui et al. 2008). This protein fixes both Cu^{+} and Cu^{2+} and seems to intervene at a later stage in detoxification because its maximum expression occurs later than for the other *cop* genes (Monchy et al. 2006a). It is a dimeric protein containing one metal-binding site per subunit. These sites have a high affinity for Cu^{2+} but can also bind Zn^{2+} and Ni^{2+}. CopH does not contain any cysteine or methionine residues but contains two histidine residues (Sendra et al. 2006).

3.1.5.5 Additional pMOL30 *cop* Genes: Evidence from Synteny and Gene Structure

As mentioned above, the function of most of the other *cop* genes remains to be fully deciphered (Table 3.1). However, additional hints can be provided by conserved synteny blocks observed during genome annotation. For instance, the *copG*, *copJ* and *copL* genes coding for cysteine-containing cytosolic proteins (CGCC motif in

CopG, ACAACH motif in CopJ and CNCC motif in CopL) (Monchy et al. 2006a) are generally closely linked to a *copF*-like gene coding for a P_{IB1}-type ATPase, suggesting that they could be collectively involved in the removal of Cu^+ from the cytosolic thiol pool (Chong et al. 2009). For instance, an orthologue of *copG*, which codes for a copper-binding protein with a thioredoxin-like fold, was identified to be transcriptionally coupled to a *copF*-like gene in *Vibrio cholerae* (Marrero et al. 2012; Bondarczuk and Piotrowska-Seget 2013). In addition, the *copO* gene is transcriptionally coupled to the *copF* gene and more generally to related genes encoding P-type ATPases of the Ag/Cu family. Most CopO proteins have a predicted length of 63–119 amino acids and contain a C-terminal histidine-rich motif beginning with a methionine residue, suggesting a role as copper chaperone for P-type ATPases. The *copK* gene, which is not frequently observed in conserved synteny blocks of copper resistance genes, is found in $ICE_{Tn4371}6067$ of *Delftia acidovorans* SPH-1 (Ryan et al. 2009; Van Houdt et al. 2009, 2013) and in conserved putative MGEs carrying the *copSRABFICDK* gene cluster found in *Stenotrophomonas maltophilia, Pseudomonas aeruginosa* and *Achromobacter piechaudii* strains. In addition, *copK* genes were also directly identified in some environments via metagenomic analysis (Jia et al. 2013).

Finally, the *copQ* gene belongs to a group of 19 homologous genes encoding for putative small (between 69 and 165 amino acids) stress responsive proteins. All have a distinctive signal peptide and are apparently only found in *Cupriavidus* and *Ralstonia* species (Janssen et al. 2010). Next to *copQ*, also *mmrQ* (*ncc* cluster pMOL30), *czcJ* (*czc* cluster pMOL30) and *mmmQ* (czc_2 cluster chromid) are part of a conserved synteny block together with metal resistance determinants. Ten of these genes were found to be highly induced in response to different heavy metals (Monsieurs et al. 2011) including *copQ* (Monchy et al. 2006a) and *czcJ* (Scherer and Nies 2009), and were even among the most induced ones, suggesting an involvement in acute stress response.

3.1.5.6 Insight in the Phenotype of *cop* Mutants

For 13 of the 21 pMOL30 *cop* genes (*copVTMKSRABDFGQE*), transposon insertion mutants are available in CH34 as well as in AE1744 (a plasmid-less derivative carrying the pMOL30 *cop* gene cluster on a broad host range cosmid) (van Aelst 2008; Monchy et al. 2006a). A slight difference in the MIC of copper between CH34 and AE1744 was observed, probably because of the additional resistance provided by other pMOL30 genes (e.g. the *silCBAD* genes). The transposon mutant library was phenotypically screened through viable counts (Mergeay 1995) in the presence of different copper concentrations (with or without pre-induction with a low copper concentration). In contrast to the MIC of metals, which is measured in liquid medium by scoring visible growth after a fixed incubation time, viable counts allow detection of more nuances in the response (Fig. 3.4). Different phenotypic variations were observed (Fig. 3.4). As expected,

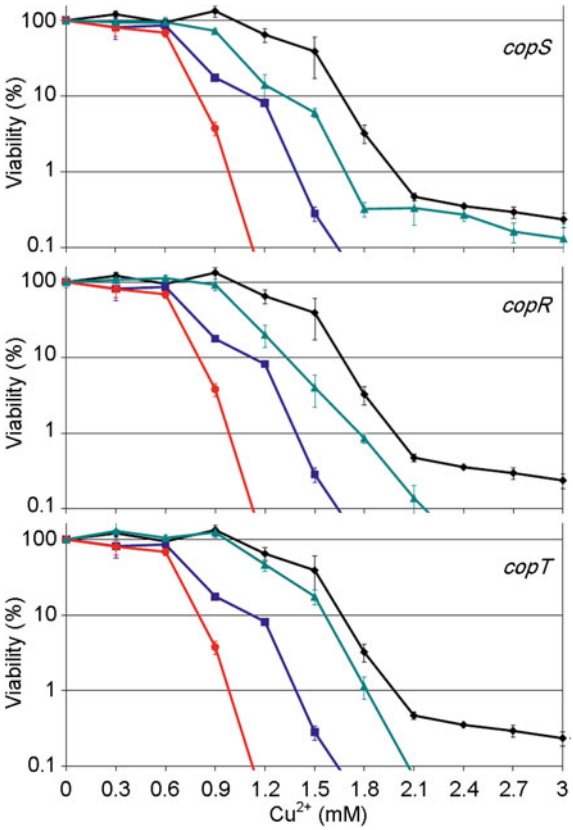

Fig. 3.4 Viability of *C. metallidurans* CH34 (*black diamond*) and its *copS, copR* or *copT* mutant (*green triangle*), AE1744 (*blue square*) and AE104 (*red circle*) on mineral Tris-buffered agar supplemented with different Cu^{2+} concentrations after pre-induction with 0.3 mM Cu^{2+}. Results are expressed as the percentage of viable counts compared with the counts on unsupplemented medium (van Aelst 2008)

cop mutants affected in the known resistance mechanisms (*copRSABCD* and *copF*) are less resistant than CH34 although still more resistant than the plasmid-free derivative AE104. However, at high copper concentrations (above the MIC for CH34), the percentage of survivors varies between different mutants. For instance, there were no CH34 *copR* mutant survivors unlike the *copS* mutant and the parental strain CH34 (Fig. 3.4) (van Aelst 2008). A similar phenotype was observed for the *copG*, *copT* and *copV* genes, which do not belong to one of the archetypical detoxification systems (Fig. 3.4) (van Aelst 2008). This phenotypic variability indicated distinct roles of particular *cop* genes in the response to copper ranging from defining the resistance level and accessory roles not directly affecting viability to surviving concentrations above the MIC. This arsenal may be advantageous to survive acute and chronic metal stress in poor environments while competing with other bacteria for the scarce nutrients.

3.1.6 Gold: An Example of Metal Reduction

C. metallidurans CH34 is able to precipitate gold by reduction of Au^{3+}-complexes to Au^{0}-particles. The process involves the formation of intermediate Au^{+}-S complexes, followed by a slow biochemically-driven reduction and intra- and extracellular deposition of metallic Au particles (Reith et al. 2009). Furthermore, bacterial biofilm communities on gold grains are dominated by *C. metallidurans* (Reith et al. 2006, 2010) and biomineralization of Au in *C. metallidurans* biofilms resulted in Au biominerals that are morphologically analogous to those observed on natural Au grains (Fairbrother et al. 2013).

Transcriptomic analysis indicated that exposure of CH34 to $AuCl_4^-$ strongly induced the *cup* region on the chromosome (*cupRAC*), the Rmet_4682-Rmet_4685 cluster on the chromid and the *cop* region on plasmid pMOL30 (*copVTMKNSRABCDIJGFOLQHEW*) (Reith et al. 2009). However, Wiesemann et al. (2013) demonstrated that plasmids pMOL28 and pMOL30 were neither required for gold resistance nor essential for the biomineralization of metallic gold particles. In addition, although the MerR-like regulator CupR, which acts as a transcriptional activator of the P_{IB1}-type ATPase CupA and the chaperone protein CupC, possesses a much lower affinity for Cu^+ than for Au^+ (Jian et al. 2009), the P_{IB1}-type ATPase CupA only affects copper and not gold resistance (Wiesemann et al. 2013). Furthermore, the *cup* cluster is only induced by $AuCl_4^-$ at concentrations above the MIC (Reith et al. 2009; Jian et al. 2009), while activation of this cluster by sublethal concentrations was only observed for Ag^+, As^{3+} and Pb^{2+} (Monsieurs et al. 2011). The Rmet_4682-Rmet_4685 cluster, which was named *dax* in Monsieurs et al. (2011) and renamed *gig* for "gold-induced genes" in Wiesemann et al. (2013), appeared not to be essential for gold resistance (Wiesemann et al. 2013). This cluster is controlled by the extracytoplasmic function (ECF) sigma factor RpoQ coded by the Rmet_4686-Rmet_4687 operon (Reith et al. 2009; Grosse et al. 2007) (with Rmet_4687 coding for a membrane protein of unknown function that could be a putative antisigma factor). The *rpoQ* gene was induced by $AuCl_4^-$ [50 and 100 μM but not 0.5 and 10 μM (Wiesemann et al. 2013; Monsieurs et al. 2011; Reith et al. 2009)] and Cu^{2+} [300 μM but not 50 μM (Grosse et al. 2007; Wiesemann et al. 2013)]. In addition, the expression of this Rmet_4682-Rmet_4685 cluster is exclusively upregulated by silver based on induction experiments using metal concentrations below the MIC (Monsieurs et al. 2011), while induction with gold only occurs after exposure to gold concentrations above the MIC. Finally, only deletion of the chromid-located $copA_2B_2C_2D_2$ determinant (see Sect. 3.1.5) led to a decrease in gold resistance (Wiesemann et al. 2013).

These results led Wiesemann et al. (2013) to conclude that up till now no gold uptake system has been identified (and putatively is not present) in *C. metallidurans*. Transportation into the cytoplasm is probably limited and reduction into gold nanoparticles occurs in the periplasm putatively by components of the respiratory chain.

3.1.7 Lead: An Interplay Between ATPase, Phosphatase and Peptidase

Lead resistance in *C. metallidurans* CH34 is mainly mediated by the pMOL30-encoded *pbrUTRABCD* (Rmet_5944-Rmet_5949) gene cluster. The *pbr* resistance operon is arranged into two divergently transcribed clusters, one coding for the MerR-family transcriptional regulator PbrR, the putative Pb^{2+} uptake protein PbrT belonging to the ILT (Iron Lead Transporter) family (Debut et al. 2006) and the MFS permease PbrU, the second codes for the P_{IB}-type ATPase PbrA, the undecaprenyl pyrophosphate phosphatase/lipoprotein signal peptidase PbrB/PbrC and the putative intracellular Pb-binding protein PbrD (Hynninen et al. 2009; Hobman et al. 2012; Taghavi et al. 2009; Borremans et al. 2001). The *pbrU* gene, downstream of *pbrT* (Taghavi et al. 2009; Monchy et al. 2007), is inactivated in CH34 by Tn*6049* insertion, but is intact in the other sequenced *C. metallidurans* strains (H1130, HMR-1, NA1, NA4 and NE12A2) (Li et al. 2013; Monsieurs et al. 2013, 2014) (see Chap. 2). The other gene flanking *pbrU*, designated *pbrV*, codes for a putative high affinity Fe^{2+}/Pb^{2+} permease. However, *pbrV* is only full-length in strain HMR-1, while partial in the other strains.

The PbrA ATPase is not specific for Pb^{2+} and also efficiently transports Zn^{2+} and Cd^{2+}. Furthermore, it can be complemented by the P_{IB}-type ATPases ZntA and CadA (Hynninen et al. 2009; Taghavi et al. 2009). The current model for lead resistance proposes that Pb^{2+} induces the expression of PbrABCD as well as ZntA and CadA, which are all able to export Pb^{2+} from the cytoplasm to the periplasm. Interaction with inorganic phosphate groups produced by PbrB-mediated dephoshorylation of undecaprenyl pyrophosphate leads to the formation of insoluble lead phosphate and prevents re-entry in the cytoplasm (Hynninen et al. 2009; Taghavi et al. 2009). PbrC appears not to be essential; however, other CH34 peptidases could putatively complement its function. One of the candidates is $pbrC_2$ located in the $pbrR_2$ *cadA* $pbrC_2$ cluster on CMGI-1 (see Chap. 2). Expression of $pbrC_2$ (and *cadA*) increased when *pbrA*, *pbrB* or *pbrD* were inactivated, probably in response to an increased intracellular Pb^{2+} concentration (Taghavi et al. 2009). Although, only very low levels of cross-regulation of the P_{pbrA} promoter by $PbrR_2$ (putatively regulating *cadA* and $pbrC_2$) and $PbrR_3$ were found (Julian et al. 2009), this indicates a synergistic interaction between both clusters and shows that acquisition of CMGI-1 improved the versatility of *C. metallidurans* CH34 towards metals (Taghavi et al. 2009).

3.1.8 Mercury: Tn*21* Family Transposons

Four mercury resistance operons are present in the genome of CH34. Two identical clusters form the accessory genes of transposons Tn*4378* and Tn*4380* (Rmet_6171-Rmet_6176) located on pMOL28 (in CMGI-28b) and pMOL30 (in

CMGI-30a), respectively (see Chap. 2). A third (partial) operon, *merRTP'*(Rmet_5990-Rmet_5992) is located near the *czc* cluster on pMOL30. The last cluster, *merRTPA* (Rmet_2312-Rmet_2315), is part of the chromosomally located CMGI-1 (Klockgether et al. 2007; Van Houdt et al. 2009; Dressler et al. 1991) (see Chap. 2).

In fact, *mer* genes conferring resistance to mercury are present in all *C. metallidurans* strains and are almost always associated with transposons or genomic islands (as is the case for many other bacteria) (Van Houdt et al. 2009, 2012; Monchy et al. 2007). It is noteworthy that the reported classification of *mer* genes on MGEs has mainly focused on clinical isolates, γ-proteobacteria and a few Firmicutes (Mindlin and Petrova 2013), while the group of mercury resistant MGEs in (metal-resistant) β-proteobacteria and environmental soil bacteria has had less attention (Mindlin and Petrova 2013).

The identical *merRTPADEurf-2* operon structures of Tn*4378* and Tn*4380* are closely related to that of Tn*501* from the *P. aeruginosa* plasmid pVS1. The cluster contains the regulatory genes *merR* and *merD*, four structural genes coding for the cytoplasmic mercuric reductase enzyme MerA, the transport system MerTP and the broad-spectrum mercury transporter MerE (Kiyono et al. 2009; Sone et al. 2013a; b), and *urf-2* coding for an unknown function. All structural proteins carry characteristic cysteine residues that mediate transport across the cytoplasmic membrane, delivery to the MerA active site and reduction (Liebert et al. 1999). All *mer* genes except *merR* are induced by Hg^{2+} and to a lesser extent by Cd^{2+}, Zn^{2+} and Pb^{2+} (Monchy et al. 2007; Monsieurs et al. 2011). In addition, the transposition modules are induced by Hg^{2+} (without an unequivocal discrimination between Tn*4738,* Tn*4380* and Tn*6050*) (Monchy et al. 2007; Monsieurs et al. 2011), which is consistent with the increased transposition frequency of mercury transposons in the presence of mercury (Sherratt et al. 1981; Kitts et al. 1982; Haritha et al. 2009). The *urf-2* gene is not observed outside of the *mer* gene cluster and has not been shown to be directly involved in resistance to mercury. It putatively codes for a signalling diguanylate phosphodiesterase with a typical EAL motif. Furthermore, the 3′ end of *urf-2* overlaps with the 5′ end of *tnpR* in the various Tn*4380* copies of sequenced *C. metallidurans* strains. In transposons of the Tn*21* lineage, a transposition-deficient integron, responsible for the acquisition and expression of antibiotic resistance genes, is inserted into the *urf-2* gene (Kholodii et al. 2003; Essa et al. 2003; Partridge et al. 2001; Liebert et al. 1999; Grinsted et al. 1990).

In strain CH34, the regulatory protein MerD (Champier et al. 2004), the transport proteins MerP (Serre et al. 2004) and MerT (Rossy et al. 2004b), and the MerP-like N-terminal extension of the mercuric reductase MerA (Rossy et al. 2004a) have been investigated. Analysis of MerD showed that although it co-regulates expression of the *mer* operon and shows a high N-terminal sequence similarity to the transcriptional regulator MerR, it does not directly bind the *mer* operator (Champier et al. 2004). Instead, MerD dissociates the Hg-MerR-*mer* operator complex allowing for the synthesis of new apo-MerR that can then bind to the *mer* operator (Silver and Hobman 2007; Champier et al. 2004). For MerP and the MerP-like N-terminal extension of MerA, it was shown that the metal-binding site consists of the highly conserved GMTCXXC sequence found in

metallochaperones and metal-transporting ATPases (Serre et al. 2004; Rossy et al. 2004a). Finally, Rossy et al. (2004b) showed that the cytoplasmic loop of MerT binds and specifically transfers Hg to MerA, in agreement with previous work on the Tn*501* mercury transport system (Morby et al. 1995). The paradigmatic regulatory protein MerR will be discussed in the regulatory section with other *C. metallidurans* regulatory proteins of the same family (Hobman 2007).

C. metallidurans strains harbour a diversity of structural *mer* genes in addition to those identified in strain CH34. For instance, *C. metallidurans* NE12 contains a *merRBTPCADEurf-2* cluster and a a *merRTPFADE* operon associated with a Tn*5053*-family transposon (Hobman et al. 1994) that is highly similar (96 % nucleotide similarity) to Tn*512* identified in *P. aeruginosa* AW54a (Petrovski and Stanisich 2010). In these variant *mer* operons, *merF*, *merE* and *merC* code for additional mercury transporters (Sone et al. 2013a; Wilson et al. 2000), and *merB* codes for an organomercurial lyase that catalyses the demethylation of a wide range of organomercurials (Parks et al. 2009; Melnick and Parkin 2007; Lafrance-Vanasse et al. 2009).

This diversity in *mer* clusters may reflect the specific history of the corresponding strains in various environments [e.g. in the case of *C. metallidurans* NE12 before being trapped in the air filter of the Kennedy Space Center Spacecraft Assembly and Encapsulation Facility II (Newcombe et al. 2008; Mijnendonckx et al. 2013)]. Some of the strains being more anthropized or more exposed to the industrial mercury cycle than others. In the environmental strain *R. pickettii* 12J, the *merTPCADB* cluster, part of a large chromosomal genomic island harbouring many metal resistance genes, contains a non-typical *merB* gene encoding for a putative alkyl-mercury lyase (27 % protein identity with MerB of pSB102 and 55 % protein identity with MerB of *Janthinobacterium* sp. Marseille).

All these observations confirm the presence of mobile genetic elements carrying mercury genes often together with other heavy metal resistance genes concentrated in chimeric genomic islands or plasmids. However, especially in the metallurgic sites where *C. metallidurans* strains were isolated, a selection pressure due to bioavailable mercuric ions is barely visible. Nevertheless, the very toxic mercury, which is much rarer than most of the toxic heavy metals, is subjected to many transformations in soil, water and air including (but not exclusively) strongly anthropized environments and nearby soils or sediments with a high content of other heavy metals (Barkay et al. 2003). The mercury cycle, which is also fed by volcanic (geothermal) releases (Boyd and Barkay 2012), is also evident from the fact that both living and dead mercury-resistant bacteria (including the plasmid-free derivative of *C. metallidurans* CH34) can re-oxidize metallic mercury released by the MerA reductase under anaerobic conditions (Colombo et al. 2014; Barkay et al. 2003).

3.1.9 Nickel: Specific Classes of HME-RND Genes

Nickel shares similar chemical and physical properties with cobalt, its neighbour in the periodic table. It is therefore not surprising to find bacterial resistance determinants that recognize and act upon both metal ions. As described previously in Sect. 3.1.4, this is the case for the inducible HME-RND-driven efflux system CnrCBA encoded by the pMOL28 operon *cnrYXHCBAT* (Rmet_6205-Rmet_6211) (Mergeay et al. 1985; Siddiqui and Schlegel 1987; Liesegang et al. 1993; Grass et al. 2000; Tibazarwa et al. 2000; Nies et al. 2006; Monsieurs et al. 2011). This determinant is mainly induced by Ni^{2+} and Co^{2+} but also by Cu^{2+} and Cd^{2+} (see Sect. 3.3.1.2) (Nies et al. 2006; Monsieurs et al. 2011). The *cnrT* gene, downstream of the *cnrCBA* genes, encodes a putative Ni^{2+} efflux transporter (Nies 2003, 2013) that belongs to the Drug/Metabolite Transporter (DMT) superfamily (see volume II). In addition, CnrT has a C-terminal domain containing 24 histidine residues that could contribute to metal ion binding. Upregulation of *cnrT* transcription was observed in the presence of Ni^{2+}, Cu^{2+} and Cd^{2+} (Monchy et al. 2007; Monsieurs et al. 2011) and the corresponding protein could induce a small degree of nickel resistance (Nies 2003).

The *cnrYXHCBAT* gene cluster on pMOL28 could originate from the acquisition of an *nccYXHCBAN*-like determinant, such as the one present on plasmid pTOM9 from *C. metallidurans* 31A, by horizontal transfer and duplication on the megaplasmid (von Rozycki and Nies 2009). While the *C. metallidurans* 31A *ncc* locus causes a high-level nickel and cobalt resistance, as well as cadmium resistance, when transferred to strain AE104 (Schmidt and Schlegel 1994), the *nccCBA* gene cluster (Rmet_6145-Rmet_6148) located on pMOL30 from *C. metallidurans* CH34 is transcriptionally silent, probably due to the lack of upstream *nccYXH* regulatory genes (Nies et al. 2006; Monsieurs et al. 2011). In addition, the presence of a frameshift in *nccB* would prevent the translation of a functional protein (Monchy et al. 2007). Expression at the protein level of a third HME-RND-driven system, the chromid-encoded NimBAC (Rmet_5682-Rmet_5677), is induced by Ni^{2+} and Co^{2+} (Auquier 2006) and even Cd^{2+} (Nies et al. 2006). However, this efflux system is not functional due to the presence of the insertion sequence element IS*Rme3* in the *nimA* gene (Janssen et al. 2010).

A second nickel resistance locus called *nre* was detected in plasmid pTOM9 from *C. metallidurans* 31A (previously designated *Achromobacter xylosoxidans* 31A) (Schmidt and Schlegel 1994; Grass et al. 2001). Among the four ORFs present in this locus, only the one encoding NreB is specifically induced by Ni^{2+} and necessary to confer low-level nickel resistance in *C. metallidurans* 31A (Grass et al. 2001). When transferred to *E. coli*, NreB reduces Ni^{2+} uptake suggesting that the protein mediates metal ion efflux. The NreB permease is a member of the MFS permeases and possesses a histidine-rich carboxy terminus contributing to maximal nickel resistance (Grass et al. 2001). NreB and its closest homologue NrsD from *Synechocystis* sp. strain PCC 6803 (Garcia-Dominguez et al. 2000) are the only known MFS proteins involved in metal ion transport. In *C. metallidurans* CH34, an

NreB orthologue encoded by Rmet_6144 was identified close to the *ncc* locus in pMOL30. This protein does not possess the characteristic histidine-rich C-terminal domain and no induction or role in nickel resistance has been demonstrated so far (Monsieurs et al. 2011).

As mentioned previously in Sect. 3.1.4, the CDF protein DmeF and the ABC-transporter AtmA are also involved in nickel resistance in *C. metallidurans* CH34 (Munkelt et al. 2004; Mikolay and Nies 2009). AtmA is most likely not an efflux system for cytoplasmic Ni^{2+} and Co^{2+} and could be involved in the transport of compounds required to repair the damage done by periplasmic cobalt or nickel ions (Mikolay and Nies 2009).

3.1.10 *Selenium: Developments About the Chromosomal* dedA *Gene*

In most environments, selenium, an essential oligo-element, predominantly occurs in its oxidized forms, selenate (SeO_4^{2-}, Se^{6+}) and selenite (SeO_3^{2-}, Se^{4+}). These bioavailable oxyanions are soluble and, at elevated concentrations, toxic and mutagenic. Selenite directly promotes oxidative stress through reaction with cellular reduced thiol-containing compounds such as glutathione (Roux et al. 2001). In *C. metallidurans* CH34, the resistance/response to selenium anions (selenite and selenite) is not associated with plasmid-borne functions.

C. metallidurans CH34 is able to resist up to 6 mM selenite and to reduce selenite to elemental red selenium, which accumulates in the cytoplasm as judged from electron microscopy and energy-dispersive X-ray analysis (Roux et al. 2001). Such cytoplasmic storage of an insoluble and non-toxic form of selenium is of interest for bioremediation (Roux et al. 2001; Saiki and Lowe 1987).

In fact, exposure of *C. metallidurans* CH34 cells to selenite induced an adaptation phase followed by a slow and then strongly increased uptake. The first process is balanced by slow assimilation into seleno-L-methionine and slow detoxification to elemental Se, after which selenite transport and reduction are activated (Avoscan et al. 2006). In contrast, exposure to selenate did not induce this adaptation phase and both selenite (transiently) and elemental Se occurred as minor species while organic selenide was the major form accumulated. Therefore, selenate mostly follows an assimilatory pathway and the reduction pathway is not activated upon selenate exposure (Sarret et al. 2005). Consequently, CH34 was not defined as being a good candidate for bioremediation of selenate-contaminated environments (Avoscan et al. 2009).

Ledgham et al. (2005) performed random Tn*5* transposon mutagenesis to isolate *C. metallidurans* CH34 mutants able to resist up to 15 mM selenite. All selenite-resistant mutants contained a Tn*5* insertion into a single chromosomal gene identified as *dedA* (Rmet_2907). The DedA protein family is a highly conserved and ancient family of membrane proteins with representatives in most sequenced

bacterial, archaeal and eukaryotic genomes (Doerrler et al. 2013). Although the functions of the DedA family proteins remain to be fully elucidated, certain bacterial DedA family members have a role in membrane homeostasis and appear to be required to maintain the proton motive force. Mutation of *dedA* affects cell division, temperature sensitivity, membrane lipid composition, envelope-stress response and proton motive force (Doerrler et al. 2013). Furthermore, the *dedA*-family genes are essential in *E. coli* (Boughner and Doerrler 2012), which points towards the presence of other DedA homologues in *C. metallidurans* CH34 (with Rmet_5938 being a possible target). According to Doerrler et al. (2013), a role of DedA in the uptake of selenite is consistent with the occurrence of DedA domains in secondary transporters of the tripartite ATP-independent periplasmic transporter (TRAP-T) family and in transporters displaying a proton symporter or antiporter activity.

3.1.11 Silver: Much More Than a Substrate for Copper-Responsive Genes

C. metallidurans CH34 carries a number of systems that are putatively involved in silver detoxification based on their homologies to known silver resistance systems. The *silDCBA* (Rmet_6133-Rmet_6139) and *cusDCBAF* (Rmet_5030-Rmet_5034) operons, which code for HME-RND-driven efflux systems, are located on pMOL30 (CMGI-30b) and the chromid, respectively (Mergeay et al. 2009; Janssen et al. 2010). The *cupRAC* (Rmet_3523-Rmet_3525) operon codes for a P_{IB1}-type ATPase and is located on the chromosome (Janssen et al. 2010). Expression of all three operons was induced after 30 min exposure to 0.25 μM Ag^+ (Monsieurs et al. 2011). Expression of the Sil and Cus proteins was also induced in *C. metallidurans* after growth in the presence of 1 μM $AgNO_3$ (Auquier 2006; Mergeay et al. 2003) and heterologous expression of SilCBA in *E. coli* GR17 increased the silver resistance of the strain 2.4-fold (Ngonlong Ekendé 2012). Furthermore, the C-terminal domain of SilB is able to bind Ag^+ (Bersch et al. 2011). In addition, eight genes from the pMOL30 *cop* cluster were transcriptionally induced by Ag^+ (Monsieurs et al. 2011; Monchy et al. 2007), including *copK* for which its product binds Ag^+ (Chong et al. 2009), *copC* and *copD* from the periplasmic detoxification system CopABCDI, the *copOFGJ* cluster with *copF* coding for a P_{IB1}-type ATPase, and *copH* coding for a paralogue of CzcE (see also volume II). Deletion of *cupA* as well as *copF* (to a lesser extent) decreased silver resistance, with a double mutant being the most sensitive (Wiesemann et al. 2013). Finally, the expression of the Rmet_4682-Rmet_4685 cluster, which was named *dax* in Monsieurs et al. (2011) and renamed *gig* for "gold-induced genes" in Wiesemann et al. (2013) (see Sect. 3.1.6), is exclusively upregulated by silver based on induction experiments using metal concentrations below the MIC (Monsieurs et al. 2011).

Proteomic data also revealed induction of the chromosomally-encoded AgrR after growth of CH34 in the presence of 1 μM $AgNO_3$ (Auquier 2006). AgrR

(Rmet_1751) is a response regulator and part of a two-component regulatory system with the histidine kinase AgrS (Rmet_1752). They share 58 and 32 % protein sequence similarity with SilR and SilS from *Salmonella enterica* serovar Typhimurium plasmid pMG101, respectively. Their co-localized target codes for an uncharacterized RND-driven efflux system (AgrABC; Rmet_1750-Rmet_1748), which shows characteristics of heavy metal (HME) as well as hydrophilic/amphiphilic compounds (HAE) efflux systems.

3.1.12 Zinc: A Pivotal Role for the RND-Driven System CzcCBA

The MIC of $ZnCl_2$ for *C. metallidurans* CH34 was determined to be 12 mM in Tris minimal medium (Mergeay et al. 1985). This high level of resistance was mainly associated to determinants located on pMOL30. The MIC of Zn^{2+} for the pMOL30-free strains AE104 and AE128 is twenty times lower than for the parental strain CH34 (Mergeay et al. 1985). The major actor is the well characterized inducible HME-RND-driven system CzcCBA (Rmet_5982-Rmet_5980) (Nies et al. 1987; Nies 1992, 1995). As previously described, this system also plays a role in the regulation of cellular cadmium and cobalt concentrations. It is induced by $Zn^{2+} \gg Cd^{2+} > Co^{2+}$ but also to a lesser extent by other cations such as Ni^{2+} and Cu^{2+} (Nies 1992; Nies et al. 2006; Monsieurs et al. 2011; van der Lelie et al. 1997; Legatzki et al. 2003a). Expression of the *czc* system is regulated by the intracellular zinc content. Furthermore, as zinc is the best inducer of *czcCBA*, the encoded protein complex might be mainly a zinc regulation system. For example, constitutive expression of CzcCBA in strain AE104 Δ*zupT* (Rmet_2621, ZupT is a zinc importer) led upon zinc starvation to the disappearance of the CzcA protein and therefore to a decrease of the metal ion efflux (Herzberg et al. 2014a). The three proteins CzcA, CzcB, and CzcC are required for full resistance to Zn^{2+}, Cd^{2+}, and Co^{2+} (Nies et al. 1989b) but CzcA alone is able to mediate a low-level metal ion resistance (Rensing et al. 1997). After reconstitution of CzcA into proteoliposomes, Zn^{2+} was transported with the highest velocity among the three different cations (Goldberg et al. 1999).

CzcCBA is the main efflux system involved in zinc resistance but a functional interplay between this tripartite complex and other transporters from *C. metallidurans* CH34 was observed. The first transporter family consists of proteins from the P-type ATPase family. Four proteins of this family are able to induce zinc resistance in different conditions, more specifically, the three P_{IB2}-type ATPases ZntA (chromid), CadA (CMGI-1 on the chromosome) and PbrA (pMOL30), and the P_{IB4}-type ATPase CzcP (pMOL30) (Scherer and Nies 2009). Heterologous expression of ZntA and CadA in a zinc-sensitive *E. coli* strain increased the resistance to Zn^{2+} and Cd^{2+} (Legatzki et al. 2003b). In strain AE104 (plasmid-less), single gene deletions of *cadA* or *zntA* had only a moderate effect on zinc and

cadmium resistance, but in a double deletion mutant zinc and cadmium resistance decreased 6-fold and 350-fold, respectively (Legatzki et al. 2003b). Neither single nor double gene deletions affected zinc resistance in the presence of CzcCBA. In the absence of CzcCBA and of the three other ATPases, ZntA is responsible for a substantial increase in zinc resistance (Scherer and Nies 2009). Deletion of *zntA* can be partially compensated by at least one of the three other ATPases. CzcP has, however, a different contribution to zinc resistance in comparison to the three P_{IB2}-type ATPases. CzcP transports zinc ions more efficiently than the three P_{IB2}-type ATPases but only when at least one of these proteins is present. Therefore, CzcP seems to play a role as "resistance enhancer" in *C. metallidurans* CH34 (Scherer and Nies 2009).

The second type of transporter that may interfere with the Czc system is represented by proteins of the CDF family. The *czcD* gene (Rmet_5979) is located downstream of the *czcCBA* cluster (Nies and Silver 1995) and mediates a low-level Zn^{2+}, Cd^{2+}, and Co^{2+} resistance when expressed in the absence of the high resistance CzcCBA system (Anton et al. 1999). CzcD is able to functionally substitute the four $P_{IB2/4}$-type ATPases and vice versa (Scherer and Nies 2009). The Zn^{2+} and Cd^{2+} efflux activity of CzcD was demonstrated by heterologous expression in a highly zinc-sensitive *E. coli* strain (Anton et al. 2004). In the presence of CzcCBA, CzcD is not essential for cation efflux in *C. metallidurans* strains (Nies et al. 1989b) but the protein is involved in the regulation of the *czc* determinant (Anton et al. 1999; Nies 1992; Grosse et al. 2004).

DmeF and FieF, two other chromosomally-encoded CDF proteins, predominantly transport Co^{2+} and Fe^{2+}, respectively. Although both proteins have a broad substrate specificity and are, for example, capable of conferring a low-level zinc resistance in a zinc-sensitive *E. coli* strain (Munkelt et al. 2004), disruption of *dmeF* or *fieF* in strains AE128 and AE104 had no influence on the tolerance to Zn^{2+} (Munkelt et al. 2004; Scherer and Nies 2009).

Zinc ions may also interfere with transport systems specific to other cations like the CnrCBA efflux system. For instance, spontaneous mutants derived from strain AE126 (containing only pMOL28) were selected on minimal medium supplemented with 0.6 to 1 mM Zn^{2+} (Collard et al. 1993). These mutants displayed constitutive expression of CnrCBA, resulting in reduced cellular accumulation of Zn^{2+} and increased resistance to zinc, cobalt and nickel (Tibazarwa et al. 2000; Collard et al. 1993)(see Sect. 3.3.1.2). In addition, full expression of the *cnr* genes in strain AE126 carrying only pMOL28 is accompanied by some resistance to zinc and even cadmium.

Finally, these studies about the resistance to zinc were complemented with efforts to make a comprehensive inventory of the zinc (and related metals) uptake systems (Kirsten et al. 2011) and their regulation, with emphasis on the *zupT* gene (Rmet_2621) (Herzberg et al. 2014a; Schmidt et al. 2014), as well as their effect on the cellular zinc pools and repository (Herzberg et al. 2014a, b).

3.1.13 Other Metals: Thallium, Caesium, Palladium and Bismuth

C. metallidurans only showed moderate changes in gene expression after exposure to thallium (around 40 upregulated genes). A surprisingly low number of differentially expressed genes were located on its megaplasmids, especially as it has been observed that the MIC of thallium is drastically reduced in the absence of pMOL28 and pMOL30. The only plasmid gene differentially expressed after thallium exposure was the *copH* gene on pMOL30.

Exposure to caesium resulted in a transcriptional response comparable with that to thallium. Correspondingly, *copH* was one of the only known metal resistance genes that was differentially expressed. This response can partially be explained by the relatively low chemotoxicity of caesium, resulting in a rather high MIC that might be more related to osmotic stress than chemical toxicity.

An even smaller effect was observed at the transcription level after exposure to palladium, resulting in about 10 upregulated genes. Nevertheless, the upregulated genes with palladium included (part of) the *cop* clusters on pMOL30 (including *copH*) and the chromid.

Exposure to bismuth had a more pronounced effect on the transcriptional profile of *C. metallidurans* CH34, resulting in a list of more than 100 differentially expressed genes. The most obvious observation is the high induction of the *ars* operon (on average more than 8-fold), an operon which had already been shown to be activated by a wide range of transition metals (Monsieurs et al. 2011). In addition, next to a large fraction of conserved hypothetical proteins, some metal resistance genes are also upregulated like *czcA*, *czcC* and *zntA*. Furthermore, in a plasmid-free derivative of CH34, mutation of *zntA* increased the sensitivity to bismuth, which is one of the first phenotypes linked to bismuth (Monchy et al. 2006b).

3.2 Applications of the *Cupriavidus* Resistance/Response to Metals

The extensive array of metal efflux/extrusion systems allows *C. metallidurans* to adapt, survive and even thrive in biotopes with a high toxic metal content. Yet surviving chronic and intense metal pollution implies the development of strategies to avoid metal re-entry such as immobilisation or sequestration.

Liquid cultures of *C. metallidurans* grown in the presence of a high levels of cadmium or zinc removed substantial amounts of these metals from the liquid via a process of bioprecipitation initiated at the onset of the stationary phase (Diels et al. 1995a, b, 1996). Crystals of otavite (cadmium carbonate) and hydrozincite (zinc hydrocarbonate: $Zn_5(CO_3)_2(OH)_6$) were observed. This first observation triggered a more thorough analysis of *C. metallidurans* liquid cultures and biofilms with special attention to polysaccharides as nucleation sites for carbonate crystals and as

contributors to the flotation behaviour of slurries containing soil, bacteria and heavy metals (Diels et al. 2009). This led to the development of various applications to efficiently remove metals from effluents, sludge and soils (Diels et al. 2009; Pumpel et al. 2003; Pumpel and Paknikar 2001).

Genetic engineering offered alternative approaches to bioremediation. On the one hand, *C. metallidurans* strains were genetically engineered to enhance their bioremediation potential. For instance, heterologous expression of eukaryotic metallothionein, a cysteine-rich protein that binds heavy metals, enhanced the immobilization capacity of *C. metallidurans* CH34 and conferred a protective effect upon tobacco plant growth in Cd^{2+}-polluted soils (Valls et al. 2000). Another example is the increased potential for mercury bioremediation of contaminated water bodies and industrial wastewater by introduction of the IncP-1β plasmid pTP6 that provides novel and additional *mer* genes (Rojas et al. 2011). On the other hand, *C. metallidurans* genes were introduced and heterologously expressed in endophytes, which assisted their host plant during phytoremediation (Lodewyckx et al. 2001; Taghavi et al. 2001; Weyens et al. 2011). These examples highlight the power of genetic engineering for environmental bioremediation.

Next to these applications, metal-induced resistance genes (mostly located on the megaplasmids from *C. metallidurans* CH34 and *C. basilensis* DS185) allowed the construction of heavy metal whole-cell biosensors[2] that are able to detect the bioavailable concentrations of cadmium, zinc, copper, chromate, cobalt, nickel, thallium, lead or mercury ions after contact with contaminated soils, waste, solids, minerals or ashes (Corbisier et al. 2002; Collard et al. 1994; Vanderlelie et al. 1994; Peitzsch et al. 1998; Nies 2000; Magrisso et al. 2008; Harms 2007). The whole-cell biosensors were constructed by fusing a *luxCDABE* reporter system to a metal-responsive promoter, thereby translating the induction in the presence of a biologically available heavy metal to a measurable bioluminescent signal. In a next step, these whole-cell biosensors were immobilized onto optical fibres and their sensitivity and storage capacity were characterised (Corbisier et al. 1999). Another type of sensor is based on the direct interaction between metal-binding proteins and heavy metal ions. For instance, the capacitance change of the MerR mercury regulatory protein (see Sect. 3.3.1.1) was detected in the presence of femto- to millimolar metal ion concentrations (Corbisier et al. 1999). Recently, a PbrR-based biosensor was also devised to measure lead concentrations (Chiu and Yang 2012).

The *C. metallidurans* biosensors have been efficiently used to measure: (i) the bioavailable metal concentration in solid wastes (such as incinerator fly ash) (Corbisier et al. 1996), soils contaminated by heavy metals (Corbisier et al. 1996; Diels et al. 1999; Almendras et al. 2009), bacteria (Gilis et al. 1998) and photoluminescent nanocrystals (Aboulaich et al. 2012); (ii) the residual concentrations of nickel and lead in soils that had been treated with additives for in situ metal immobilization (Tibazarwa et al. 2001; Geebelen et al. 2003); (iii) the biological

[2]For a general evaluation of the microbial reporters designed to assay metal bioavailability and their possible use in environmental remediation see Magrisso et al. (2008).

accumulation of nickel in specific parts of important agricultural crops such as maize and potato (Tibazarwa et al. 2001); (iv) the runoff from zinc-coated materials and outdoor structures, which is an important source for zinc dispersion in the environment (Heijerick et al. 2002); and (v) the intracellular Pb^{2+} content in human embryonic kidney cells (Chiu and Yang 2012).

Finally, it should be mentioned that metal resistance determinants from *C. metallidurans* CH34 proved to be very versatile tools to study gene dissemination in soil environments (the problematic nature of spreading recombinant genes in the environment) (Top et al. 1990, 1995; Smets et al. 2003), to detect new broad host range plasmids in the rhizosphere or in polluted soils (Top et al. 1994; Van der Auwera et al. 2009; van Elsas et al. 1998), and to evaluate conjugational plasmid transfer between Proteobacteria and Actinobacteria (Margesin and Schinner 1997).

3.3 Lessons Learned from the Transcriptome Response to Metals and Insights into the Regulatory Network

The development of microarrays has opened up a wide area of new opportunities for the field of molecular biology. The availability of microarrays to study gene expression in *C. metallidurans* CH34 has significantly contributed to the identification of new metal resistance regions in its genome. Transcriptome analysis initially focused on the pMOL28- and pMOL30-encoded metal resistance regions as both megaplasmids were known to contain a significant number of metal resistance determinants. At a later stage, the importance of metal resistance regions located on the chromosome and the chromid was recognized (Legatzki et al. 2003b; Grosse et al. 2004; Munkelt et al. 2004; Nies et al. 2006; Monsieurs et al. 2011; Taghavi et al. 2009). A crucial issue in the analysis of transcriptome data is to distinguish differentially expressed genes truly contributing to resistance to a specific metal from those for which the biologically relevant contribution to resistance has not been validated with experimental data (e.g. knock-out mutants). For instance, the transcriptional response of *C. metallidurans* exposed to different individual metals clearly showed an overlapping set of genes being activated, for which it was unclear whether they were contributing to the resistance phenotype or whether they were being activated as a result of crosstalk from other regulators in the highly interconnected transcriptional regulatory network. Moreover, the metal concentration used, and as such the relative toxicity of it, and the time point of analysis are crucial factors in the interpretation of the transcriptomic response. Indeed, when taking the MIC as a measure of relative toxicity, significant differences are observed when comparing gene expression below and above the MIC. Significant differences in gene expression levels were also obtained at 10 min compared to 30 min after metal exposure, suggesting distinct biological responses at both time points.

The first observation on the complex regulatory network and corresponding regulatory proteins underlying heavy metal resistance is more extensively discussed

in the first part of Sect. 3.3. In the second part of Sect. 3.3, the impact of relative metal toxicity and the time-wise evolution of gene expression after exposure to metals are described in detail.

3.3.1 Regulatory Functions

In the most simplistic view, transcriptional regulatory proteins can be split into two different groups: (1) sigma factors that bind to promoter regions and attract an RNA polymerase to start the expression of the corresponding coding regions and (2) regulatory proteins that are responsible for fine-tuning the transcriptional regulation by either enhancing the binding of the sigma factor with a specific region or inhibiting its binding. The regulatory proteins involved in metal resistance are described in Sect. 3.3.1.1, while the large family of sigma factors in *C. metallidurans* CH34 is described in Sect. 3.3.1.2.

3.3.1.1 Regulatory Proteins

Most of the regulatory proteins involved in heavy metal resistance have been identified by the adjacent location of their coding sequences to genes known to be important for heavy metal resistance. Those metal resistance regulatory proteins can largely be subdivided into cytoplasmic regulators, i.e. transcriptional regulators that directly sense the presence of metal ions in the cytoplasm, and two-component regulatory systems, i.e. a regulatory system consisting of a transmembrane sensing protein able to detect metal ions in the periplasm that interacts with the corresponding transcriptional regulatory protein.

MerR-like Regulators

The largest class of cytoplasmic regulators in *C. metallidurans* CH34 involved in heavy metal resistance is the group of MerR-like regulatory proteins, of which the mercuric ion sensing MerR protein is the archetype (Hobman et al. 2005). MerR-like regulators (mostly activators) specifically recognize promoters with an unusually long spacer of 19 or 20 bp between the −10 and −35 regions, and contain cysteine amino acids essential in metal binding and activation of gene expression (Brown et al. 2003; Chen et al. 2007; Helmann et al. 1990; Shewchuk et al. 1989a, b, c). However, it has been suggested that there is some flexibility in the exact binding site recognized by MerR-like regulators since MerR-like activators in *C. metallidurans* CH34 could heterologously activate non-cognate MerR family promoters from other organisms (Julian et al. 2009).

Four mercury resistance operons are present in the CH34 genome (see Sect. 3.1.8). With the exception of the incomplete *mer* operon on pMOL30, each

operon carries a *merR* gene flanked by a mercury reductase gene (*merA*), which reduces the Hg^{2+} ion to volatile Hg(0), together with the auxiliary genes *merP* and *merT* (respectively present in the periplasm and inner membrane for transporting Hg^{2+} into to cytoplasm) (reviewed in Barkay et al. 2003). In most *mer* operons a co-regulator encoded by the *merD* gene is also flanking the *merR* regulatory gene, however, no direct binding of the MerD protein with any of the promoters in the *mer* operon could be detected (Champier et al. 2004).

In the absence of Hg^{2+}, MerR binds as a homodimer to the divergent promoter region of the *merTPAD* operon and the *merR* gene, thereby slightly repressing the transcription of these genes (reviewed in Hobman et al. 2005). However, the MerR regulatory protein is hypersensitive to Hg^{2+}, and upon the presence of Hg^{2+} in the cytoplasm, one Hg^{2+} ion will bind to three essential cysteine residues of the MerR homodimer, two cysteines from one monomer and one from the other (Helmann et al. 1990; Utschig et al. 1995). This will lead to an allosteric underwinding of the promoter DNA thereby aligning the −35 and −10 recognition sites and outweighing the effect of the prolonged spacer between both recognition sites such that RNA polymerase can bind to those sites and activate transcription (Frantz and O'Halloran 1990; Ansari et al. 1992, 1995; Heltzel et al. 1990).

A second subgroup of MerR-like regulators are the PbrR regulatory proteins, of which three copies exist in strain CH34 i.e. the $pbrR_1$ regulator of the *pbr* operon on pMOL30, the $pbrR_2$ on CMGI-1 and the chromosomally-encoded $pbrR_3$. Remarkably and in contrast with the complete *mer* operons, the *pbrR* genes do not always flank the same metal resistance genes. The $pbrR_1$ gene (Rmet_5946) is part of the *pbrUTRABCD* metal resistance region on pMOL30 and is shown to regulate this *pbr* operon (Borremans et al. 2001; Brown et al. 2003), as *pbrRTU* and *pbrABCD* are transcribed via a *merR*-like promoter (Borremans et al. 2001; Taghavi et al. 2009). The $pbrR_2$ gene (Rmet_2302) regulates the flanking efflux ATPase *cadA* gene and a *pbrC* homologue. In contrast, the chromosomally-encoded $PbrR_3$ (Rmet_3456) regulates the chromid-located *zntA* gene encoding for a P_{IB2}-type ATPase (Taghavi et al. 2009). Despite the high homology between all three PbrR regulators, cross-regulation between the three regulatory proteins seems to be rather limited. Indeed, using a β-galactosidase assay where the promoter of the pMOL30 *pbrA* gene was cloned into a reporter plasmid and linked to the *lacZ* gene, Julian et al. (2009) could detect a transcriptional activation in strain CH34 after adding Pb (II). By contrast, no transcriptional activation could be observed under the same conditions when inserting this plasmid carrying the P_{pbrA}-*lacZ* fusion into strain AE104 i.e. a *C. metallidurans* strain lacking pMOL28 and pMOL30. This suggests that transcriptional activation in the first experiment is due to the $pbrR_1$ regulator, since the chromosomally located $pbrR_2$ and $pbrR_3$ are not capable of activating the pMOL30 *pbrA* promoter in a plasmid-free strain. As such, only low cross-talk between the three *pbrR* regulators could be observed at least in the case of the pMOL30 *pbrA* promoter.

The DNA-binding characteristics of $PbrR_1$ have been shown to be similar to the transcriptional activation by MerR proteins, since shortening the internal spacer between the −10 and −35 region in the *pbrA* promoter to 18 bp leads to an

increased expression profile from this promoter even in the absence of Pb^{2+} (Hobman et al. 2012). Similar to other MerR regulatory proteins, it has been shown that specific cysteine amino acids in the $PbrR_1$ regulatory protein (C14, C79 and C134) are essential for Pb^{2+} sensing and activation of the *pbrA* promoter region (Hobman et al. 2012). Additionally, the metal-binding specificity of the $PbrR_2$ protein has been studied in detail, showing that this regulatory protein binds Pb^{2+} a 1000-fold more selective than a wide range of other metal ions (Chen et al. 2005). This specific binding would be the consequence of a specific geometry allowing highly specific binding of Pb^{2+} while preventing the binding with other metal ions (Chen et al. 2007).

A last representative of the MerR-like regulators linked to metal resistance, is the *cupR* gene located on the chromosome. CupR regulates resistance to metal ions by transcriptionally activating the neighbouring *cupA* gene coding for a P_{IB1}-type ATPase and auxiliary chaperone genes like *cupC*. The CupR protein is an ortholog of the GolS regulator in *Salmonella* (Pontel et al. 2007) and the CueR regulatory protein in *E. coli* (Stoyanov and Brown 2003; Stoyanov et al. 2001). CupR in *C. metallidurans* CH34 is important for resistance to monovalent copper (Cu^+), but also seems to have a high affinity for Au^+ (Jian et al. 2009), which is in line with the observation of Changela et al. (2003) who reported a very high sensitivity of the *E. coli* CueR towards Cu^+ as well as Ag^+ and Au^{3+} (Stoyanov et al. 2001; Stoyanov and Brown 2003).

ArsR-like Regulators

Six representatives of the ArsR family of regulatory proteins have been found in *C. metallidurans* CH34, evenly divided over the chromosome and the chromid, however, only one of them (ArsR, Rmet_0333) has been shown to be important for heavy metal resistance and is part of the complete *ars* operon *arsRIC*$_2$*BC*$_1$*HP* (see Sect. 3.1.1). Remarkably, the frequently occurring *arsD* gene, encoding a minor secondary regulatory protein (Silver and Phung 1996), is lacking in the *C. metallidurans* CH34 *ars* operon. Despite upregulation of the *ars* operon being observed in *C. metallidurans* CH34 after exposure to As^{3+} as well as As^{5+}, it has been shown that ArsR could only efficiently bind to the highly toxic arsenite (As^{3+}) but could not bind to the phosphate analogue arsenate (As^{5+}) (Zhang et al. 2009). This can be explained by the fact that when As^{5+} enters the bacterial cell, a small fraction is converted to As^{3+} by the arsenate reductase ArsC. Once ArsR can bind to arsenite, the ArsR repressor dissociates from the promoter region of the *ars* operon containing two arsenate reductases ($ArsC_1$ and $ArsC_2$) able to further convert As^{5+} to As^{3+}, which can then be transported to the exterior of the cell by the ArsB arsenate permease. However, the *ars* operon is not only transcriptionally induced after exposure to arsenite and arsenite, but also to a wide range of other metals like Pb^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, and to a lesser extent also Se^{6+}. However, when comparing those expression studies with in vitro binding studies of ArsR with a wide range of metals, the correlation between both observations is not straightforward (Zhang

et al. 2009). Studies using an *arsR* knock-out mutant in other organisms like *Synechocystis* sp. PCC 6803 have shown that those mutants seem to be hypersensitive to metals like Ni^{2+}, Cu^{2+} and Cd^{2+} (Sanchez-Riego et al. 2014).

Two-Component Regulators

In contrast with the one-component regulatory systems such as the MerR-like and ArsR-like regulatory proteins, where the metal ion first needs to enter the cytoplasm before transcriptional activation occurs, two-component systems have the advantage of being able to sense the metal ions in the periplasm. Some of the 56 two-component regulatory systems in *C. metallidurans* CH34 (Janssen et al. 2010) have been shown to be important for heavy metal resistance e.g. the *copRS* (Monchy et al. 2007; Mergeay et al. 2003) and *czcRS* systems (van der Lelie et al. 1997; Nies 1992; Grosse et al. 1999, 2004; Monchy et al. 2007), while for others a link with heavy metal resistance has been suggested e.g. *agrRS* (Mergeay et al. 2003), *hmzRS* (Van Houdt et al. 2009), *zniRS* and *zneRS* (Nies et al. 2006). Despite the fact that two-component systems have been shown to play a crucial role in metal resistance against a wide range of heavy metals (Monchy et al. 2007; Wiesemann et al. 2013), less is known about metal binding affinities and specificities of those two-component regulatory systems. Indeed, as already stated (Hobman et al. 2007), metal responsive cytoplasmic transcription factors are becoming well understood, however, the working mechanisms and binding characteristics of two-component systems, and cross-talk between regulators in heavy metal resistance is less intensively studied.

Most of the regulators of the known metal resistance mechanisms in *C. metallidurans* can be grouped in one of the different classes described above. As mentioned in the introduction of this paragraph, the majority of the regulatory proteins have been identified by adjacency of their coding sequences to known metal resistance clusters. However, some of the regions predicted to be important for heavy metal resistance (e.g. *nim*, *sil* or *hmy*) do not have a regulator in the neighbouring regions. It must be noted that most of those regions do not show differential expression after exposure to a wide range of heavy metals (Monsieurs et al. 2011). Only *nimB* (Rmet_5682) was moderately upregulated after exposure to Cu^{2+} and Co^{2+}, *silA* (Rmet_6136) with Cu^{2+}, *hmyC* (Rmet_4120) with Pb^{2+}, Bi^{2+} and Sr^{2+}, and *hmyB* (Rmet_4121) with Sr^{2+}. For these regions, additional studies will be required to investigate whether they are under control of a specific regulator.

Additionally, it should be noted that the regulatory control of some metal resistance regions needs to be further deciphered. An intriguing example within this context is the transcriptional regulation of the *chr* region, which is responsible for resistance to chromate and present in duplicate in strain CH34 i.e. one copy on pMOL28 and a second one on the chromid. It was already hypothesized that the *chrB* and *chrI* genes are involved in this regulation, suggesting a role in transcriptional activation for *chrB* and a repressive role for *chrI* (Juhnke et al. 2002). However, additional studies are needed to further elucidate this mechanism.

3.3.1.2 Sigma Factors and the Regulation of *cnr/ncc* Nickel Resistance

All bacterial transcriptional processes are initiated by the binding of a sigma factor to the promoter region of a gene. In many cases this transcriptional process is initiated by the housekeeping sigma factor σ^{70}. *C. metallidurans* CH34 has undergone different gene duplication processes including that of the housekeeping sigma factor. Indeed, two RpoD (σ^{70}) copies are present in CH34 i.e. *rpoD1* (Rmet_2606) on the chromosome and *rpoD2* (Rmet_4661) on the chromid (Janssen et al. 2010; von Rozycki and Nies 2009). Comparative genomic analysis suggests that the *rpoD1* gene encodes the native housekeeping factor, while the *rpoD2* gene has been acquired via horizontal gene transfer as it shows good homology with the σ^{70} factor present in *Bordetella* and *Burkholderia* species (Janssen et al. 2010).

In addition to these housekeeping sigma factors, bacteria use alternate sigma factors to fine-tune their transcriptomic profile or to activate subsets of genes in response to a wide range of stresses. As such, *C. metallidurans* CH34 has acquired 18 different sigma factors (compared to 8 in *E. coli*). Even more impressive is the portion of extracytoplasmic function (ECF) sigma factors, as there are up to 11 different ECF sigma factors (Nies 2004; Grosse et al. 2007). This subset of ECF sigma factors is important in the context of metal resistance as they can respond to extracytoplasmic signals. Of these ECF sigma factors, five have been shown to be important for heavy metal resistance and metabolism i.e. RpoI (Rmet_1120) (control of staphyloferrin siderophore biosynthesis), RpoJ (Rmet_4499), RpoK (Rmet_4001), RpoE (Rmet_2425) and CnrH (Rmet_6207).

The latter ECF sigma factor regulates the *cnr* region on megaplasmid pMOL28 and can deal with resistance to cobalt, nickel and zinc (Liesegang et al. 1993; Grass et al. 2000; Tibazarwa et al. 2000). Control of the activity of CnrH occurs via two transmembrane proteins i.e. the anti-sigma factor CnrY and the periplasmic sensor CnrX. In absence of Ni^{2+} and Co^{2+}, the sigma factor CnrH is silenced via the binding of CnrY. However, upon binding of the periplasmic sensor CnrX with Ni^{2+} or Co^{2+} in the periplasm, CnrH is released by CnrY, and the transcription of the *cnr* region can proceed. In a recent paper, Maillard et al. (2014) described the structural interaction between CnrH and CnrY, and identified a hydrophobic knot that is responsible for the release of CnrH from CnrY in the presence of nickel or cobalt. Important to notice within this context is the uncertainty on the promoter regions controlling the *cnr* region. While Grass et al. (2000) suggested the presence of two possible tricistronic transcripts i.e. *cnrYXH* and *cnrCBA*, Tibazarwa et al. (2000) suggested a dicistronic *cnrYX* operon and tetracistronic *cnrHCBA* operon, requiring the promoter of the latter operon to be located inside the *cnrX* gene.

3.3.2 Cross-Regulation

In the most straightforward implementation of transcriptional regulation of heavy metal resistance, each single metal ion would be recognized by one specific sensor

protein, which in turn would activate a dedicated resistance mechanism (Hobman 2007; Hobman et al. 2005, 2007). However, observing the transcriptional response of *C. metallidurans* CH34 when exposed to a wide range of metals and metalloids over time courses has clearly showed that this does not correspond to the biological reality, either on the plasmid (Monchy et al. 2007) or on the chromosome (Monsieurs et al. 2011). Indeed, based on the correlation between gene expression profiles for different individual metals as well as by calculating the number of overlapping genes between different metal responses, a complex interplay between different metal responses was observed. This corresponds with the observation made by Hobman et al. (2007) when exposing *E. coli* to a wide range of metals, thereby stating as conclusion that most of those metal response regulons are not discrete but rather overlap between different metal exposures.

This cross-regulation of gene expression by different metals might be the result of different events. First, it should be noted that many metalloproteins do not bind with their cognate effector metal when faced with mixtures of metals (Waldron and Robinson 2009). This might not be surprising as the chemistry of metal ions makes it such that biological systems may have low metal ion specificity (Hobman et al. 2007). This had already been shown e.g. in *E. coli* where it was shown that CueR could not only bind, but also activate expression in response to Cu^{+}, as well as Ag^{+} and Au^{3+} (Stoyanov et al. 2001; Stoyanov and Brown 2003) and is also illustrated in *C. metallidurans* CH34 for the ArsR regulatory protein that seems to be able to bind a wide range of metals and derepress transcription (Zhang et al. 2009), while the corresponding *ars* operon is only shown to be important for As resistance. Exposing *C. metallidurans* to a specific metal might induce expression of certain gene clusters for which it is not clear whether their gene products contribute to the metal resistance phenotype, or whether it is caused by an erroneous binding of the metal with a metalloprotein.

Secondly, an increase in the concentration of one specific metal in the environment might lead to an elevated concentration in the cytoplasm, thereby influencing the balance of the intracellular metal ion pool (Kaur et al. 2006). This might result in a shift in occupation of metal binding sites by binding of the excessive metal ion to the general metal binding proteins and forcing the trace elements in the cytoplasm to bind with highly specific metal binding proteins, thereby upregulating genes related to specific metal resistance characteristics. Similarly, some toxic metals might have a higher affinity for an occupied essential metal binding site in a protein, and as such displace the essential metal, which would increase the intracellular free metal ion pool. This might for example be the case for the exposure of *C. metallidurans* CH34 to gold. The pMOL30 *cop* cluster was only upregulated at toxic gold concentrations (Reith et al. 2009), which might be explained by occupation of the majority of non-specific metal binding domains by gold causing a shift of copper and other *cop* activating metals to their corresponding highly metal-specific sensors (Monsieurs et al. 2011).

Thirdly, this cross-regulation might be explained by the flexibility of regulatory systems at the transcriptional level to recognize transcription factor binding sites slightly deviating from their consensus site. Indeed, many regulatory proteins

known to be linked to heavy metal resistance are highly conserved i.e. they display a high level of homology. This is, for example, the case for a cluster of CopR-like two-component regulators i.e. the pMOL30-encoded $CopR_1$ and $CzcR_1$, and the chromid-encoded $CopR_2$, $CzcR_2$ and AgrR. All five regulators show a high level of homology at the protein level and are likely to recognize the same type of regulatory motifs. This implies that upon activation these regulatory proteins are not only transcriptionally activating their cognate operons but also other metal resistance operons normally regulated by other regulatory proteins.

Similarly, the sensor proteins of two-component systems, responsible for detecting the presence of metals in two-component regulatory system, might show alike homology as the regulatory proteins. These sensory proteins show flexibility in the corresponding regulatory protein they can activate via phosphorylation. A proof of principle of this type of cross-regulation is the *czc* system in pMOL30. It was shown that upon deletion of the *czcS* gene encoding the histidine kinase, two other homologous histidine kinases were able to take over this function, thereby ensuring the transcriptional activation by the regulatory protein CzcR via binding with the promoter regions of *czcP* and *czcN* (Grosse et al. 1999; Scherer and Nies 2009).

3.3.3 Dose- and Time-Dependency of the Response

In order to allow an objective comparison between different metal ion expression profiles, most data were obtained after metal exposure to similar conditions i.e. an exposure of 30 min to a metal concentration approaching ~50 % of the MIC. Moreover, for most of the metals only one speciation of the metal has been tested. Below, some small-scale results concerning the potential influence of these factors will be discussed.

3.3.3.1 Concentration Effect

As mentioned above, most metal response expression profiles are obtained using one specific metal concentration below the MIC. However, for exposure to Au^{3+}, five different data sets are available: a ten-minute exposure to 10, 50 and 100 μM, and a thirty-minute exposure to 0.5 and 50 μM (Monsieurs et al. 2011; Reith et al. 2009). First of all, a clear effect of the increase in gold concentration can be observed: the total number of upregulated genes at 10 min augments with an increasing concentration of gold i.e. the number of upregulated genes is 53, 127 and 330 for 10, 50 and 100 μM, respectively. A similar trend in differentially upregulated genes can be observed for both concentrations at 30 min (increasing from 59 at 0.5 μM to 136 genes at 50 μM). Moreover, the obtained fold changes increase proportionally to the applied concentration. From a functional point of view, when looking at metal response gene clusters expressed at high gold concentrations (50

and 100 μM) versus lower ones (10 and 0.5 μM), genes related to oxidative stress (e.g. the *ohrR* regulon) and glutaredoxin/glutathione metabolism are exclusively upregulated at high gold concentrations. Additionally, when comparing the different metal responses at the level of functional classes (COGs), a very clear activation of the chaperone proteins (proteins classified in COG class O) is observed with increasing Au^{3+} concentrations: where at 0.5 μM this consisted only of 3 % of all upregulated genes, this percentage increased to 14 % for 100 μM and around 21 % for both observations at 50 μM. Similarly, the increase in the number of upregulated genes is confirmed by the increase of the number of genes related to the transcriptional process, which increased from 1 % at 0.5 μM to around 13 % at 50 μM.

At a smaller scale, an expression study using qPCR has been applied for the pMOL30 *cop* cluster (Monchy et al. 2006a), leading to results that are deviating from the gold transcriptomic data. The pMOL30 *cop* genes of *C. metallidurans* CH34 were exposed to different concentrations of Cu^{2+}, and the fold change in expression after 30 min exposure was measured using qPCR. Instead of obtaining fold changes that increase proportionally with the applied concentration a dramatic decrease in fold change was observed once the copper concentration added to the growth media reached the MIC.

Based on the two data sets described above, it is very difficult to predict what the exact effect of increasing metal concentrations, even exceeding the MIC, will have on the transcriptional process in *C. metallidurans*. Indeed, where the fold changes still increase for gold when applying a concentration more than 100 fold higher than the MIC, the opposite is observed for the pMOL30 *cop* genes with exposure to copper concentrations just below the MIC. As these conclusions are only based on a limited number of experiments, a more thorough analysis of the transcriptomic profile above the MIC is required, using a wider range of metals in a well-defined experimental setup.

3.3.3.2 Time Effect

Transcriptional regulation is a dynamic progress, and the type of genes upregulated after exposure to metals will also depend on the time point when gene expression is measured. Datasets assessing transcriptional regulation at different time points after exposure to metals in *C. metallidurans* are limited. However, data from a transcriptomics experiment measuring genome-wide transcription after exposure to gold and a qPCR experiment assessing the expression levels after exposure to different concentrations of copper allow us to derive some general trends.

Looking at the overlap of the upregulated genes at 10 and 30 min after exposure to 50 μM gold, around 50 % of the upregulated genes are the same at both time points. It is not clear based on the functional classification of these genes, which physiological effect can possibly explain this shift in gene expression, as for both time points the same functional classes are over- or underrepresented. However, when focusing on metal-specific genes (i.e. genes with a limited amount of

orthologs in related bacteria), a change in two opposite directions can be observed. First, for some metal-specific clusters, a clear increase of gene expression was observed at 30 min compared with 10 min. An illustrative example for this phenomenon is the *cop* cluster on pMOL30. Despite the fact that this region is already partially upregulated at 10 min (9 out of 19 genes; average fold change 3), a significant increase is observed at 30 min (14 out of 17 genes; average fold change 9). As a second example, Rmet_4682-Rmet_4686 is already fully activated at 10 min with an average fold change of 9, however increasing to an average fold change of 46 at 30 min. In contrast, some of the known metal-resistance clusters are upregulated immediately after exposure of *C. metallidurans* to gold, but the effect decreases with time (almost no differential expression detected after 30 min). Examples of this behaviour are the *mer*-cluster on Tn*4378* (average fold change of $\sim$3 at 10 min vs. 1.70 at 30 min), the *pbr* operon on the chromid (fold change $\sim$6 at 10 min vs. $\sim$2 at 30 min) and the *ars* operon on CMGI-1 (average of 13 at 10 min vs. 1.80 at 30 min).

This might indicate that the first reaction of the bacterium is a general stress response, thereby activating a wide range of metal resistance mechanisms, where a more specific response is seen at 30 min. This hypothesis is confirmed by qPCR data (Monchy et al. 2006a), following up the transcriptional profile over time of the pMOL30 *cop* genes after exposure to Cu^{2+}. Indeed, where after 10 min an increase in fold change can already be observed for most *cop* genes, this effect is even more pronounced after 30 min.

On the other hand, in the case of the Tn*4378 mer* operon, MerR activation of expression is balanced by MerD acting to dissociate Hg^{2+}-bound MerR from P_{merT} as a feedback control mechanism (Champier et al. 2004). Many transcription studies have concentrated on activation of gene expression in response to metals, but being able to switch off expression is as important for the organism to maintain a dynamic response to changing internal and external conditions.

3.4 Concluding Remarks

C. metallidurans, with type strain CH34 used as case study, is highly adapted to hostile environments with a high burden of multiple metals. Because of these environments, it is not unexpected that its metal resistance determinants and their transcriptional network would be similarly complicated, overlapping, robust and to contain a degree of redundancy. Human engineering solutions to critically important safety and survival systems also build in system duplication, robustness and redundancy, to avoid catastrophic failure in case components of the system fail to operate correctly. In the real world (rather than the lab) it is very unlikely that this strain would ever be exposed to single toxic metal species at 99.9 % purity in controlled growth conditions, it is more likely that exposure would be to multiple metals maybe in different forms, alongside other physical insults (pH, water activity, nutrient, temperature).

References

Aboulaich A, Tilmaciu CM, Merlin C, Mercier C, Guilloteau H, Medjahdi G, Schneider R (2012) Physicochemical properties and cellular toxicity of (poly)aminoalkoxysilanes-functionalized ZnO quantum dots. Nanotechnology 23(33):335101

Ajees AA, Marapakala K, Packianathan C, Sankaran B, Rosen BP (2012) Structure of an As(III) S-adenosylmethionine methyltransferase: insights into the mechanism of arsenic biotransformation. Biochemistry 51(27):5476–5485

Almendras M, Carballa M, Diels L, Vanbroekhoven K, Chamy R (2009) Prediction of heavy metals mobility and bioavailability in contaminated soil using sequential extraction and biosensors. J Environ Eng 135(9):839–844

Ansari AZ, Bradner JE, O'Halloran TV (1995) DNA-bend modulation in a repressor-to-activator switching mechanism. Nature 374(6520):371–375

Ansari AZ, Chael ML, O'Halloran TV (1992) Allosteric underwinding of DNA is a critical step in positive control of transcription by Hg-MerR. Nature 355(6355):87–89

Anton A, Grosse C, Reissmann J, Pribyl T, Nies DH (1999) CzcD is a heavy metal ion transporter involved in regulation of heavy metal resistance in *Ralstonia* sp. strain CH34. J Bacteriol 181(22):6876–6881

Anton A, Weltrowski A, Haney CJ, Franke S, Grass G, Rensing C, Nies DH (2004) Characteristics of zinc transport by two bacterial cation diffusion facilitators from *Ralstonia metallidurans* CH34 and *Escherichia coli*. J Bacteriol 186(22):7499–7507

Ash M-R, Chong LX, Maher MJ, Hinds MG, Xiao Z, Wedd AG (2011) Molecular basis of the cooperative binding of Cu(I) and Cu(II) to the CopK protein from *Cupriavidus metallidurans* CH34. Biochemistry 50:9237–9247

Auquier V (2006) Identification et caractérisation de protéines membranaires impliquées dans les systèmes de résistance aux métaux lourds chez *Cupriavidus metallidurans* CH34. Université Libre de Bruxelles, Brussels, Belgium

Avoscan L, Carrière M, Proux O, Sarret G, Degrouard J, Covès J, Gouget B (2009) Enhanced selenate accumulation in *Cupriavidus metallidurans* CH34 does not trigger a detoxification pathway. Appl Environ Microbiol 75(7):2250–2252

Avoscan L, Collins R, Carrière M, Gouget B, Covès J (2006) Seleno-L-methionine is the predominant organic form of selenium in *Cupriavidus metallidurans* CH34 exposed to selenite or selenate. Appl Environ Microbiol 72(9):6414–6416

Barkay T, Miller SM, Summers AO (2003) Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Rev 27(2–3):355–384

Bersch B, Derfoufi KM, De Angelis F, Auquier V, Ekende EN, Mergeay M, Ruysschaert JM, Vandenbussche G (2011) Structural and metal binding characterization of the C-terminal metallochaperone domain of membrane fusion protein SilB from *Cupriavidus metallidurans* CH34. Biochemistry 50(12):2194–2204

Bersch B, Favier A, Schanda P, van Aelst S, Vallaeys T, Covès J, Mergeay M, Wattiez R (2008) Molecular structure and metal-binding properties of the periplasmic CopK protein expressed in *Cupriavidus metallidurans* CH34 during copper challenge. J Mol Biol 380(2):386–403

Bondarczuk K, Piotrowska-Seget Z (2013) Molecular basis of active copper resistance mechanisms in Gram-negative bacteria. Cell Biol Toxicol 29(6):397–405

Borremans B, Hobman JL, Provoost A, Brown NL, Van der Lelie D (2001) Cloning and functional analysis of the *pbr* lead resistance determinant of *Ralstonia metallidurans* CH34. J Bacteriol 183(19):5651–5658

Boughner LA, Doerrler WT (2012) Multiple deletions reveal the essentiality of the DedA membrane protein family in *Escherichia coli*. Microbiology 158(Pt 5):1162–1171

Boyd ES, Barkay T (2012) The mercury resistance operon: from an origin in a geothermal environment to an efficient detoxification machine. Front Microbiol 3:349

Brown NL, Stoyanov JV, Kidd SP, Hobman JL (2003) The MerR family of transcriptional regulators. FEMS Microbiol Rev 27(2–3):145–163

Champier L, Duarte V, Michaud-Soret I, Covès J (2004) Characterization of the MerD protein from *Ralstonia metallidurans* CH34: a possible role in bacterial mercury resistance by switching off the induction of the mer operon. Mol Microbiol 52(5):1475–1485

Changela A, Chen K, Xue Y, Holschen J, Outten CE, O'Halloran TV, Mondragon A (2003) Molecular basis of metal-ion selectivity and zeptomolar sensitivity by CueR. Science 301 (5638):1383–1387

Chen P, Greenberg B, Taghavi S, Romano C, van der Lelie D, He C (2005) An exceptionally selective lead(II)-regulatory protein from *Ralstonia metallidurans*: development of a fluorescent lead(II) probe. Angew Chem Int Ed Engl 44(18):2715–2719

Chen PR, Wasinger EC, Zhao J, van der Lelie D, Chen LX, He C (2007) Spectroscopic insights into lead(II) coordination by the selective lead(II)-binding protein PbrR691. J Am Chem Soc 129(41):12350–12351

Chiu TY, Yang DM (2012) Intracellular Pb^{2+} content monitoring using a protein-based Pb^{2+} indicator. Toxicol Sci 126(2):436–445

Chong LX, Ash MR, Maher MJ, Hinds MG, Xiao ZG, Wedd AG (2009) Unprecedented binding cooperativity between Cu(I) and Cu(II) in the copper resistance protein CopK from *Cupriavidus metallidurans* CH34: implications from structural studies by NMR spectroscopy and X-Ray crystallography. J Am Chem Soc 131(10):3549–3564

Collard JM, Corbisier P, Diels L, Dong Q, Jeanthon C, Mergeay M, Taghavi S, van der Lelie D, Wilmotte A, Wuertz S (1994) Plasmids for heavy metal resistance in *Alcaligenes eutrophus* CH34: mechanisms and applications. FEMS Microbiol Rev 14(4):405–414

Collard JM, Provoost A, Taghavi S, Mergeay M (1993) A new type of *Alcaligenes eutrophus* CH34 zinc resistance generated by mutations affecting regulation of the *cnr* cobalt-nickel resistance system. J Bacteriol 175(3):779–784

Colombo MJ, Ha J, Reinfelder JR, Barkay T, Yee N (2014) Oxidation of Hg(0) to Hg(II) by diverse anaerobic bacteria. Chem Geol 363:334–340

Corbisier P, Diels L, Mergeay M (2002) Fused genes and their use for determining the presence of metals or of xenobiotic compounds. Patent EP0573500/US 5786162 A

Corbisier P, Thiry E, Diels L (1996) Bacterial biosensors for the toxicity assessment of solid wastes. Environ Toxicol Water Qual 11(3):171–177

Corbisier P, van der Lelie D, Borremans B, Provoost A, de Lorenzo V, Brown NL, Lloyd JR, Hobman JL, Csoregi E, Johansson G, Mattiasson B (1999) Whole cell- and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples. Anal Chim Acta 387(3):235–244

Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: a sequence logo generator. Genome Res 14(6):1188–1190

Crossman LC, Chaudhuri RR, Beatson SA, Wells TJ, Desvaux M, Cunningham AF, Petty NK, Mahon V, Brinkley C, Hobman JL, Savarino SJ, Turner SM, Pallen MJ, Penn CW, Parkhill J, Turner AK, Johnson TJ, Thomson NR, Smith SG, Henderson IR (2010) A commensal gone bad: complete genome sequence of the prototypical enterotoxigenic *Escherichia coli* strain H10407. J Bacteriol 192(21):5822–5831

de Castro E, Sigrist CJ, Gattiker A, Bulliard V, Langendijk-Genevaux PS, Gasteiger E, Bairoch A, Hulo N (2006) ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. Nucleic Acids Res 34 (Web Server issue):W362–W365

Debut AJ, Dumay QC, Barabote RD, Saier MH Jr (2006) The iron/lead transporter superfamily of Fe/Pb^{2+} uptake systems. J Mol Microbiol Biotechnol 11(1–2):1–9

Diaz-Perez C, Cervantes C, Campos-Garcia J, Julian-Sanchez A, Riveros-Rosas H (2007) Phylogenetic analysis of the chromate ion transporter (CHR) superfamily. FEBS J 274 (23):6215–6227

Diels L, De Smet M, Hooyberghs L, Corbisier P (1999) Heavy metals bioremediation of soil. Mol Biotechnol 12(2):149–158

Diels L, Dong QH, van der Lelie D, Baeyens W, Mergeay M (1995a) The *czc* operon of *Alcaligenes eutrophus* CH34: from resistance mechanism to the removal of heavy metals. J Ind Microbiol 14(2):142–153

Diels L, Van Roy S, Leysen R, Mergeay M (1996) Heavy metal bioprecipitation by *Alcaligenes eutrophus* CH34 immobilized in a membrane bioreactor. Int Biodeter Biodegr 37(3–4):239

Diels L, Van Roy S, Somers K, Willems I, Doyen W, Mergeay M, Springael D, Leysen R (1995b) The use of bacteria immobilized in tubular membrane reactors for heavy metal recovery and degradation of chlorinated aromatics. J Membr Sci 100(3):249–258

Diels L, Van Roy S, Taghavi S, Van Houdt R (2009) From industrial sites to environmental applications with *Cupriavidus metallidurans*. Anton Leeuw Int J G 96(2):247–258

Djoko KY, Xiao Z, Wedd AG (2008) Copper resistance in *E. coli*: the multicopper oxidase PcoA catalyzes oxidation of copper(I) in Cu(I)Cu(II)-PcoC. ChemBioChem 9(10):1579–1582

Doerrler WT, Sikdar R, Kumar S, Boughner LA (2013) New functions for the ancient DedA membrane protein family. J Bacteriol 195(1):3–11

Dressler C, Kues U, Nies DH, Friedrich B (1991) Determinants encoding resistance to several heavy metals in newly isolated copper-resistant bacteria. Appl Environ Microbiol 57 (11):3079–3085

Essa AM, Julian DJ, Kidd SP, Brown NL, Hobman JL (2003) Mercury resistance determinants related to Tn*21*, Tn*1696*, and Tn*5053* in enterobacteria from the preantibiotic era. Antimicrob Agents Chemother 47(3):1115–1119

Fairbrother L, Etschmann B, Brugger J, Shapter J, Southam G, Reith F (2013) Biomineralization of gold in biofilms of *Cupriavidus metallidurans*. Environ Sci Technol 47(6):2628–2635

Frantz B, O'Halloran TV (1990) DNA distortion accompanies transcriptional activation by the metal-responsive gene-regulatory protein MerR. Biochemistry 29(20):4747–4751

Garcia-Dominguez M, Lopez-Maury L, Florencio FJ, Reyes JC (2000) A gene cluster involved in metal homeostasis in the cyanobacterium *Synechocystis* sp. strain PCC 6803. J Bacteriol 182(6):1507–1514

Geebelen W, Adriano DC, van der Lelie D, Mench M, Carleer R, Clijsters H, Vangronsveld J (2003) Selected bioavailability assays to test the efficacy of amendment-induced immobilization of lead in soils. Plant Soil 249(1):217–228

Gilis A, Corbisier P, Baeyens W, Taghavi S, Mergeay M, van der Lelie D (1998) Effect of the siderophore alcaligin E on the bioavailability of Cd to *Alcaligenes eutrophus* CH34. J Ind Microbiol Biotechnol 20(1):61–68

Gilis A, Khan MA, Cornelis P, Meyer JM, Mergeay M, van der Lelie D (1996) Siderophore-mediated iron uptake in *Alcaligenes eutrophus* CH34 and identification of *aleB* encoding the ferric iron-alcaligin E receptor. J Bacteriol 178(18):5499–5507

Gilmour MW, Thomson NR, Sanders M, Parkhill J, Taylor DE (2004) The complete nucleotide sequence of the resistance plasmid R478: defining the backbone components of incompatibility group H conjugative plasmids through comparative genomics. Plasmid 52(3):182–202

Goldberg M, Pribyl T, Juhnke S, Nies DH (1999) Energetics and topology of CzcA, a cation/proton antiporter of the resistance-nodulation-cell division protein family. J Biol Chem 274(37):26065–26070

Grass G, Fan B, Rosen BP, Lemke K, Schlegel HG, Rensing C (2001) NreB from *Achromobacter xylosoxidans* 31A Is a nickel-induced transporter conferring nickel resistance. J Bacteriol 183 (9):2803–2807

Grass G, Grosse C, Nies DH (2000) Regulation of the *cnr* cobalt and nickel resistance determinant from *Ralstonia* sp. strain CH34. J Bacteriol 182(5):1390–1398

Grinsted J, de la Cruz F, Schmitt R (1990) The Tn*21* subgroup of bacterial transposable elements. Plasmid 24(3):163–189

Grosse C, Anton A, Hoffmann T, Franke S, Schleuder G, Nies DH (2004) Identification of a regulatory pathway that controls the heavy-metal resistance system Czc via promoter czcNp in *Ralstonia metallidurans*. Arch Microbiol 182(2–3):109–118

Grosse C, Friedrich S, Nies DH (2007) Contribution of extracytoplasmic function sigma factors to transition metal homeostasis in *Cupriavidus metallidurans* strain CH34. J Mol Microbiol Biotechnol 12(3–4):227–240

Grosse C, Grass G, Anton A, Franke S, Santos AN, Lawley B, Brown NL, Nies DH (1999) Transcriptional organization of the *czc* heavy-metal homeostasis determinant from *Alcaligenes eutrophus*. J Bacteriol 181(8):2385–2393

Haritha A, Sagar KP, Tiwari A, Kiranmayi P, Rodrigue A, Mohan PM, Singh SS (2009) MrdH, a novel metal resistance determinant of *Pseudomonas putida* KT2440, is flanked by metal-inducible mobile genetic elements. J Bacteriol 191(19):5976–5987

Harms H (2007) Biosensing of Heavy Metals. In: Nies D, Silver S (eds) Molecular microbiology of heavy metals, vol 6., Microbiology monographsSpringer, Berlin, pp 143–157

Heijerick DG, Janssen CR, Karlen C, Wallinder IO, Leygraf C (2002) Bioavailability of zinc in runoff water from roofing materials. Chemosphere 47(10):1073–1080

Helmann JD, Ballard BT, Walsh CT (1990) The MerR metalloregulatory protein binds mercuric ion as a tricoordinate, metal-bridged dimer. Science 247(4945):946–948

Heltzel A, Lee IW, Totis PA, Summers AO (1990) Activator-dependent preinduction binding of sigma-70 RNA polymerase at the metal-regulated *mer* promoter. Biochemistry 29(41):9572–9584

Henne KL, Nakatsu CH, Thompson DK, Konopka AE (2009a) High-level chromate resistance in *Arthrobacter* sp. strain FB24 requires previously uncharacterized accessory genes. BMC Microbiol 9:199

Henne KL, Turse JE, Nicora CD, Lipton MS, Tollaksen SL, Lindberg C, Babnigg G, Giometti CS, Nakatsu CH, Thompson DK, Konopka AE (2009b) Global proteomic analysis of the chromate response in *Arthrobacter* sp. strain FB24. J Proteome Res 8(4):1704–1716

Herzberg M, Bauer L, Nies DH (2014a) Deletion of the *zupT* gene for a zinc importer influences zinc pools in *Cupriavidus metallidurans* CH34. Metallomics 6(3):421–436

Herzberg M, Dobritzsch D, Helm S, Baginsky S, Nies DH (2014b) The zinc repository of *Cupriavidus metallidurans*. Metallomics 6(11):2157–2165

Hobman J, Kholodii G, Nikiforov V, Ritchie DA, Strike P, Yurieva O (1994) The sequence of the *mer* operon of pMER327/419 and transposon ends of pMER327/419, 330 and 05. Gene 146(1):73–78

Hobman JL (2007) MerR family transcription activators: similar designs, different specificities. Mol Microbiol 63(5):1275–1278

Hobman JL, Crossman L (2015) Bacterial antimicrobial metal ion resistance. J Med Microbiol 64:471–497

Hobman JL, Julian DJ, Brown NL (2012) Cysteine coordination of Pb(II) is involved in the PbrR-dependent activation of the lead-resistance promoter, PpbrA, from *Cupriavidus metallidurans* CH34. BMC Microbiol 12:109

Hobman JL, Wilkie J, Brown NL (2005) A design for life: prokaryotic metal-binding MerR family regulators. Biometals 18(4):429–436

Hobman JL, Yamamoto K, Oshima T (2007) Transcriptomic responses of bacterial cells to sublethal metal ion stress. In: Nies D, Silver S (eds) Molecular microbiology of heavy metals, vol 6., Microbiology monographsSpringer, Berlin, pp 73–115

Hynninen A, Touze T, Pitkanen L, Mengin-Lecreulx D, Virta M (2009) An efflux transporter PbrA and a phosphatase PbrB cooperate in a lead-resistance mechanism in bacteria. Mol Microbiol 74(2):384–394

Janssen PJ, Van Houdt R, Moors H, Monsieurs P, Morin N, Michaux A, Benotmane MA, Leys N, Vallaeys T, Lapidus A, Monchy S, Médigue C, Taghavi S, McCorkle S, Dunn J, van der Lelie D, Mergeay M (2010) The complete genome sequence of *Cupriavidus metallidurans* strain CH34, a master survivalist in harsh and anthropogenic environments. PLoS ONE 5(5): e10433

Jia S, Wang Z, Zhang XX, Liu B, Li W, Cheng S (2013) Metagenomic analysis of cadmium and copper resistance genes in activated sludge of a tannery wastewater treatment plant. J Environ Biol 34:375–380

Jian X, Wasinger EC, Lockard JV, Chen LX, He C (2009) Highly sensitive and selective gold(I) recognition by a metalloregulator in *Ralstonia metallidurans*. J Am Chem Soc 131(31):10869–10871

Juhnke S, Peitzsch N, Hubener N, Grosse C, Nies DH (2002) New genes involved in chromate resistance in *Ralstonia metallidurans* strain CH34. Arch Microbiol 179(1):15–25

Julian DJ, Kershaw CJ, Brown NL, Hobman JL (2009) Transcriptional activation of MerR family promoters in *Cupriavidus metallidurans* CH34. Anton Leeuw Int J G 96(2):149–159

Kaur A, Pan M, Meislin M, Facciotti MT, El-Gewely R, Baliga NS (2006) A systems view of haloarchaeal strategies to withstand stress from transition metals. Genome Res 16(7):841–854

Kholodii G, Mindlin S, Petrova M, Minakhina S (2003) Tn*5060* from the Siberian permafrost is most closely related to the ancestor of Tn*21* prior to integron acquisition. FEMS Microbiol Lett 226(2):251–255

Kirsten A, Herzberg M, Voigt A, Seravalli J, Grass G, Scherer J, Nies DH (2011) Contributions of five secondary metal uptake systems to metal homeostasis of *Cupriavidus metallidurans* CH34. J Bacteriol 193(18):4652–4663

Kitts P, Symington L, Burke M, Reed R, Sherratt D (1982) Transposon-specified site-specific recombination. Proc Natl Acad Sci USA 79(1):46–50

Kiyono M, Sone Y, Nakamura R, Pan-Hou H, Sakabe K (2009) The MerE protein encoded by transposon Tn*21* is a broad mercury transporter in *Escherichia coli*. FEBS Lett 583 (7):1127–1131

Klockgether J, Wurdemann D, Reva O, Wiehlmann L, Tummler B (2007) Diversity of the abundant pKLC102/PAGI-2 family of genomic islands in *Pseudomonas aeruginosa*. J Bacteriol 189(6):2443–2459

Lafrance-Vanasse J, Lefebvre M, Di Lello P, Sygusch J, Omichinski JG (2009) Crystal structures of the organomercurial lyase MerB in its free and mercury-bound forms: insights into the mechanism of methylmercury degradation. J Biol Chem 284(2):938–944

Ledgham F, Quest B, Vallaeys T, Mergeay M, Covès J (2005) A probable link between the DedA protein and resistance to selenite. Res Microbiol 156(3):367–374

Legatzki A, Franke S, Lucke S, Hoffmann T, Anton A, Neumann D, Nies DH (2003a) First step towards a quantitative model describing Czc-mediated heavy metal resistance in *Ralstonia metallidurans*. Biodegradation 14(2):153–168

Legatzki A, Grass G, Anton A, Rensing C, Nies DH (2003b) Interplay of the Czc system and two P-type ATPases in conferring metal resistance to *Ralstonia metallidurans*. J Bacteriol 185(15):4354–4361

Li LG, Cai L, Zhang T (2013) Genome of *Cupriavidus* sp. HMR-1, a Heavy Metal-Resistant Bacterium. Genome Announc 1(1):e00202–e00212

Liebert CA, Hall RM, Summers AO (1999) Transposon Tn*21*, flagship of the floating genome. Microbiol Mol Biol Rev 63(3):507–522

Liesegang H, Lemke K, Siddiqui RA, Schlegel HG (1993) Characterization of the inducible nickel and cobalt resistance determinant *cnr* from pMOL28 of *Alcaligenes eutrophus* CH34. J Bacteriol 175(3):767–778

Lodewyckx C, Taghavi S, Mergeay M, Vangronsveld J, Clijsters H, van der Lelie D (2001) The effect of recombinant heavy metal-resistant endophytic bacteria on heavy metal uptake by their host plant. Int J Phytoremediat 3(2):173–187

Magrisso S, Erel Y, Belkin S (2008) Microbial reporters of metal bioavailability. Microb Biotechnol 1(4):320–330

Maillard AP, Girard E, Ziani W, Petit-Hartlein I, Kahn R, Covès J (2014) The crystal structure of the anti-sigma factor CnrY in complex with the sigma factor CnrH shows a new structural class of anti-sigma factors targeting extracytoplasmic function sigma factors. J Mol Biol 426(12):2313–2327

Margesin R, Schinner F (1997) Heavy metal resistant *Arthrobacter* sp.—a tool for studying conjugational plasmid transfer between gram-negative and gram-positive bacteria. J Basic Microbiol 37(3):217–227

Marrero K, Sanchez A, Gonzalez LJ, Ledon T, Rodriguez-Ulloa A, Castellanos-Serra L, Perez C, Fando R (2012) Periplasmic proteins encoded by VCA0261-0260 and VC2216 genes together with *copA* and *cueR* products are required for copper tolerance but not for virulence in *Vibrio cholerae*. Microbiology 158(Pt 8):2005–2016

Melnick JG, Parkin G (2007) Cleaving mercury-alkyl bonds: a functional model for mercury detoxification by MerB. Science 317(5835):225–227

Mergeay M (1995) Heavy metal resistances in microbial ecosystems. In: Akkermans ADL, Van Elsas JD, De Bruijn FJ (eds) Molecular microbial ecology manual. Springer, The Netherlands, pp 439–455

Mergeay M, Gerits J, Houba C (1978a) Transmissible resistance factor to cobalt in a hydrogen-utilizing *Pseudomonas*. C R Seances Soc Biol Fil 172(3):575–579

Mergeay M, Houba C, Gerits J (1978b) Extrachromosomal inheritance controlling resistance to cadmium, cobalt, copper and zinc ions: evidence from curing a *Pseudomonas*. Arch Int Physiol Biochim Biophys 86(2):440–442

Mergeay M, Monchy S, Janssen P, Van Houdt R, Leys N (2009) Megaplasmids in *Cupriavidus* genus and metal resistance. In: Schwartz E (ed) Microbial megaplasmids, vol 11., Microbiology monographsSpringer, Berlin, pp 209–238

Mergeay M, Monchy S, Vallaeys T, Auquier V, Benotmane A, Bertin P, Taghavi S, Dunn J, van der Lelie D, Wattiez R (2003) *Ralstonia metallidurans*, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes. FEMS Microbiol Rev 27(2–3):385–410

Mergeay M, Nies D, Schlegel HG, Gerits J, Charles P, Van Gijsegem F (1985) *Alcaligenes eutrophus* CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. J Bacteriol 162(1):328–334

Mijnendonckx K, Provoost A, Ott CM, Venkateswaran K, Mahillon J, Leys N, Van Houdt R (2013) Characterization of the survival ability of *Cupriavidus metallidurans* and *Ralstonia pickettii* from space-related environments. Microb Ecol 65(2):347–360

Mikolay A, Nies DH (2009) The ABC-transporter AtmA is involved in nickel and cobalt resistance of *Cupriavidus metallidurans* strain CH34. Anton Leeuw Int J G 96(2):183–191

Mindlin S, Petrova M (2013) Mercury resistance transposons. In: Roberts AP, Mullany P (eds) Bacterial integrative mobile genetic elements. Landes Biosciences, Austin, USA, pp 33–52

Monchy S, Benotmane MA, Janssen P, Vallaeys T, Taghavi S, van der Lelie D, Mergeay M (2007) Plasmids pMOL28 and pMOL30 of *Cupriavidus metallidurans* are specialized in the maximal viable response to heavy metals. J Bacteriol 189(20):7417–7425

Monchy S, Benotmane MA, Wattiez R, van Aelst S, Auquier V, Borremans B, Mergeay M, Taghavi S, van der Lelie D, Vallaeys T (2006a) Transcriptomic and proteomic analyses of the pMOL30-encoded copper resistance in *Cupriavidus metallidurans* strain CH34. Microbiology 152(Pt 6):1765–1776

Monchy S, Vallaeys T, Bossus A, Mergeay M (2006b) Metal transport ATPase genes from *Cupriavidus metallidurans* CH34: a transcriptomic approach. Int J Environ Anal Chem 86(9):677–692

Monsieurs P, Mijnendonckx K, Provoost A, Venkateswaran K, Ott CM, Leys N, Van Houdt R (2014) Genome sequences of *Cupriavidus metallidurans* strains NA1, NA4, and NE12, isolated from space equipment. Genome Announc 2(4):e00719–e00714

Monsieurs P, Moors H, Van Houdt R, Janssen PJ, Janssen A, Coninx I, Mergeay M, Leys N (2011) Heavy metal resistance in *Cupriavidus metallidurans* CH34 is governed by an intricate transcriptional network. Biometals 24(6):1133–1151

Monsieurs P, Provoost A, Mijnendonckx K, Leys N, Gaudreau C, Van Houdt R (2013) Genome sequence of *Cupriavidus metallidurans* Strain H1130, isolated from an invasive human infection. Genome Announc 1(6):e01051–e01013

Morby AP, Hobman JL, Brown NL (1995) The role of cysteine residues in the transport of mercuric ions by the Tn*501* MerT and MerP mercury-resistance proteins. Mol Microbiol 17(1):25–35

Munkelt D, Grass G, Nies DH (2004) The chromosomally encoded cation diffusion facilitator proteins DmeF and FieF from *Wautersia metallidurans* CH34 are transporters of broad metal specificity. J Bacteriol 186(23):8036–8043

Munzinger M, Taraz K, Budzikiewicz H (1999) Staphyloferrin B, a citrate siderophore of *Ralstonia eutropha*. Zeitschrift Fur Naturforschung C-a J Biosci 54(11):867–875

Newcombe DA, La Duc MT, Vaishampayan P, Venkateswaran K (2008) Impact of assembly, testing and launch operations on the airborne bacterial diversity within a spacecraft assembly facility clean-room. Int J Astrobiol 7(3 & 4):223–236

Ngonlong Ekendé EC (2012) Towards a better understanding of bacterial resistance to heavy metal ions: the case of the Sil and Zne systems from *Cupriavidus metallidurans* CH34. Université Libre de Bruxelles, Brussels, Belgium

Nies A, Nies DH, Silver S (1989a) Cloning and expression of plasmid genes encoding resistances to chromate and cobalt in *Alcaligenes eutrophus*. J Bacteriol 171(9):5065–5070

Nies D, Mergeay M, Friedrich B, Schlegel HG (1987) Cloning of plasmid genes encoding resistance to cadmium, zinc, and cobalt in *Alcaligenes eutrophus* CH34. J Bacteriol 169(10):4865–4868

Nies DH (1992) CzcR and CzcD, gene products affecting regulation of resistance to cobalt, zinc, and cadmium (*czc* system) in *Alcaligenes eutrophus*. J Bacteriol 174(24):8102–8110

Nies DH (1995) The cobalt, zinc, and cadmium efflux system CzcABC from *Alcaligenes eutrophus* functions as a cation-proton antiporter in *Escherichia coli*. J Bacteriol 177 (10):2707–2712

Nies DH (2000) Heavy metal-resistant bacteria as extremophiles: molecular physiology and biotechnological use of *Ralstonia* sp CH34. Extremophiles 4(2):77–82

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev 27 (2–3):313–339

Nies DH (2004) Incidence and function of sigma factors in *Ralstonia metallidurans* and other bacteria. Arch Microbiol 181(4):255–268

Nies DH (2007) Bacterial Transition Metal Homeostasis. In: Nies DH, Silver S (eds) Molecular microbiology of heavy metals, vol 6., Microbiology monographsSpringer, Berlin, pp 117–142

Nies DH (2013) RND efflux pumps for metal cations. In: Yu EW, Zhang Q, Brown MH (eds) Microbial efflux pumps. Caister Academic Press, Norfolk

Nies DH, Koch S, Wachi S, Peitzsch N, Saier MH Jr (1998) CHR, a novel family of prokaryotic proton motive force-driven transporters probably containing chromate/sulfate antiporters. J Bacteriol 180(21):5799–5802

Nies DH, Nies A, Chu L, Silver S (1989b) Expression and nucleotide sequence of a plasmid-determined divalent cation efflux system from *Alcaligenes eutrophus*. Proc Natl Acad Sci USA 86(19):7351–7355

Nies DH, Rehbein G, Hoffmann T, Baumann C, Grosse C (2006) Paralogs of genes encoding metal resistance proteins in *Cupriavidus metallidurans* strain CH34. J Mol Microbiol Biotechnol 11(1–2):82–93

Nies DH, Silver S (1989) Plasmid-determined inducible efflux is responsible for resistance to cadmium, zinc, and cobalt in *Alcaligenes eutrophus*. J Bacteriol 171(2):896–900

Nies DH, Silver S (1995) Ion efflux systems involved in bacterial metal resistances. J Ind Microbiol 14(2):186–199

Ohtake H, Cervantes C, Silver S (1987) Decreased chromate uptake in *Pseudomonas fluorescens* carrying a chromate resistance plasmid. J Bacteriol 169(8):3853–3856

Parks JM, Guo H, Momany C, Liang L, Miller SM, Summers AO, Smith JC (2009) Mechanism of Hg-C protonolysis in the organomercurial lyase MerB. J Am Chem Soc 131(37):13278–13285

Partridge SR, Brown HJ, Stokes HW, Hall RM (2001) Transposons Tn*1696* and Tn*21* and their integrons In4 and In2 have independent origins. Antimicrob Agents Chemother 45(4): 1263–1270

Peitzsch N, Eberz G, Nies DH (1998) *Alcaligenes eutrophus* as a bacterial chromate sensor. Appl Environ Microbiol 64(2):453–458

Petit-Haertlein I, Girard E, Sarret G, Hazemann JL, Gourhant P, Kahn R, Covès J (2010) Evidence for conformational changes upon copper binding to *Cupriavidus metallidurans* CzcE. Biochemistry 49(9):1913–1922

Petrovski S, Stanisich VA (2010) Tn*502* and Tn*512* are res site hunters that provide evidence of resolvase-independent transposition to random sites. J Bacteriol 192(7):1865–1874

Pontel LB, Audero ME, Espariz M, Checa SK, Soncini FC (2007) GolS controls the response to gold by the hierarchical induction of *Salmonella*-specific genes that include a CBA efflux-coding operon. Mol Microbiol 66(3):814–825

Pumpel T, Macaskie LE, Finlay JA, Diels L, Tsezos M (2003) Nickel removal from nickel plating waste water using a biologically active moving-bed sand filter. Biometals 16(4):567–581

Pumpel T, Paknikar KM (2001) Bioremediation technologies for metal-containing wastewaters using metabolically active microorganisms. Adv Appl Microbiol 48:135–169

Qin J, Rosen BP, Zhang Y, Wang G, Franke S, Rensing C (2006) Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. Proc Natl Acad Sci U S A 103(7):2075–2080

Reith F, Etschmann B, Grosse C, Moors H, Benotmane MA, Monsieurs P, Grass G, Doonan C, Vogt S, Lai B, Martinez-Criado G, George GN, Nies DH, Mergeay M, Pring A, Southam G, Brugger J (2009) Mechanisms of gold biomineralization in the bacterium *Cupriavidus metallidurans*. Proc Natl Acad Sci USA 106(42):17757–17762

Reith F, Fairbrother L, Nolze G, Wilhelmi O, Clode PL, Gregg A, Parsons JE, Wakelin SA, Pring A, Hough R, Southam G, Brugger J (2010) Nanoparticle factories: biofilms hold the key to gold dispersion and nugget formation. Geology 38(9):843–846

Reith F, Rogers SL, McPhail DC, Webb D (2006) Biomineralization of gold: biofilms on bacterioform gold. Science 313(5784):233–236

Rensing C, Grass G (2003) *Escherichia coli* mechanisms of copper homeostasis in a changing environment. FEMS Microbiol Rev 27(2–3):197–213

Rensing C, Pribyl T, Nies DH (1997) New functions for the three subunits of the CzcCBA cation-proton antiporter. J Bacteriol 179(22):6871–6879

Rojas LA, Yanez C, Gonzalez M, Lobos S, Smalla K, Seeger M (2011) Characterization of the metabolically modified heavy metal-resistant *Cupriavidus metallidurans* strain MSR33 generated for mercury bioremediation. PLoS ONE 6(3):e17555

Rossy E, Champier L, Bersch B, Brutscher B, Blackledge M, Covès J (2004a) Biophysical characterization of the MerP-like amino-terminal extension of the mercuric reductase from *Ralstonia metallidurans* CH34. J Biol Inorg Chem 9(1):49–58

Rossy E, Seneque O, Lascoux D, Lemaire D, Crouzy S, Delangle P, Covès J (2004b) Is the cytoplasmic loop of MerT, the mercuric ion transport protein, involved in mercury transfer to the mercuric reductase? FEBS Lett 575(1–3):86–90

Roux M, Covès J (2002) The iron-containing superoxide dismutase of *Ralstonia metallidurans* CH34. FEMS Microbiol Lett 210(1):129–133

Roux M, Sarret G, Pignot-Paintrand I, Fontecave M, Covès J (2001) Mobilization of selenite by *Ralstonia metallidurans* CH34. Appl Environ Microbiol 67(2):769–773

Ryan MP, Pembroke JT, Adley CC (2009) Novel Tn*4371*-ICE like element in *Ralstonia pickettii* and genome mining for comparative elements. BMC Microbiol 9:242

Saiki M, Lowe TP (1987) Selenium in aquatic organisms from subsurface agricultural drainage water, San Joaquin Valley, California. Arch Environ Contam Toxicol 16(6):657–670

San Martin-Uriz P, Mirete S, Alcolea PJ, Gomez MJ, Amils R, Gonzalez-Pastor JE (2014) Nickel-resistance determinants in *Acidiphilium* sp. PM identified by genome-wide functional screening. PLoS One 9(4):e95041

Sanchez-Riego AM, Lopez-Maury L, Florencio FJ (2014) Genomic responses to arsenic in the cyanobacterium *Synechocystis* sp. PCC 6803. PLoS ONE 9(5):e96826

Sandegren L, Linkevicius M, Lytsy B, Melhus A, Andersson DI (2012) Transfer of an *Escherichia coli* ST131 multiresistance cassette has created a *Klebsiella pneumoniae*-specific plasmid associated with a major nosocomial outbreak. J Antimicrob Chemother 67(1):74–83

Sarret G, Avoscan L, Carriere M, Collins R, Geoffroy N, Carrot F, Covès J, Gouget B (2005) Chemical forms of selenium in the metal-resistant bacterium *Ralstonia metallidurans* CH34 exposed to selenite and selenate. Appl Environ Microbiol 71(5):2331–2337

Sarret G, Favier A, Covès J, Hazemann JL, Mergeay M, Bersch B (2010) CopK from *Cupriavidus metallidurans* CH34 binds Cu(I) in a tetrathioether site: characterization by X-ray absorption and NMR spectroscopy. J Am Chem Soc 132(11):3770–3777

Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13(11):2844–2854

Scherer J, Nies DH (2009) CzcP is a novel efflux system contributing to transition metal resistance in *Cupriavidus metallidurans* CH34. Mol Microbiol 73(4):601–621

Schmidt C, Schwarzenberger C, Grosse C, Nies DH (2014) FurC regulates expression of zupT for the central zinc importer ZupT of *Cupriavidus metallidurans*. J Bacteriol 196(19):3461–3471

Schmidt T, Schlegel HG (1994) Combined nickel-cobalt-cadmium resistance encoded by the *ncc* locus of *Alcaligenes xylosoxidans* 31A. J Bacteriol 176(22):7045–7054

Schmidt T, Stoppel RD, Schlegel HG (1991) High-level nickel resistance in *Alcaligenes xylosoxydans* 31A and *Alcaligenes eutrophus* KTO2. Appl Environ Microbiol 57(11):3301–3309

Sendra V, Cannella D, Bersch B, Fieschi F, Menage S, Lascoux D, Covès J (2006) CopH from *Cupriavidus metallidurans* CH34. A novel periplasmic copper-binding protein. Biochemistry 45(17):5557–5566

Serre L, Rossy E, Pebay-Peyroula E, Cohen-Addad C, Covès J (2004) Crystal structure of the oxidized form of the periplasmic mercury-binding protein MerP from *Ralstonia metallidurans* CH34. J Mol Biol 339(1):161–171

Sherratt D, Arthur A, Burke M (1981) Transposon-specified, site-specific recombination systems. Cold Spring Harb Symp Quant Biol 45(Pt 1):275–281

Shewchuk LM, Helmann JD, Ross W, Park SJ, Summers AO, Walsh CT (1989a) Transcriptional switching by the MerR protein: activation and repression mutants implicate distinct DNA and mercury(II) binding domains. Biochemistry 28(5):2340–2344

Shewchuk LM, Verdine GL, Nash H, Walsh CT (1989b) Mutagenesis of the cysteines in the metalloregulatory protein MerR indicates that a metal-bridged dimer activates transcription. Biochemistry 28(15):6140–6145

Shewchuk LM, Verdine GL, Walsh CT (1989c) Transcriptional switching by the metalloregulatory MerR protein: initial characterization of DNA and mercury (II) binding activities. Biochemistry 28(5):2331–2339

Siddiqui RA, Benthin K, Schlegel HG (1989) Cloning of pMOL28-encoded nickel resistance genes and expression of the genes in *Alcaligenes eutrophus* and *Pseudomonas* spp. J Bacteriol 171(9):5071–5078

Siddiqui RA, Schlegel HG (1987) Plasmid pMOL28-mediated inducible nickel resistance in *Alcaligenes eutrophus* strain CH34. FEMS Microbiol Lett 43(1):9–13

Silver S, Hobman JL (2007) Mercury microbiology: resistance systems, environmental aspects, methylation, and human health. In: Nies DH, Silver S (eds) Molecular microbiology of heavy metals, vol 6., Microbiology monographsSpringer, Berlin, pp 357–370

Silver S, Phung LT (1996) Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol 50:753–789

Smets BF, Morrow JB, Pinedo CA (2003) Plasmid introduction in metal-stressed, subsurface-derived microcosms: plasmid fate and community response. Appl Environ Microbiol 69(7):4087–4097

Sone Y, Nakamura R, Pan-Hou H, Itoh T, Kiyono M (2013a) Role of MerC, MerE, MerF, MerT, and/or MerP in resistance to mercurials and the transport of mercurials in *Escherichia coli*. Biol Pharm Bull 36(11):1835–1841

Sone Y, Nakamura R, Pan-Hou H, Sato MH, Itoh T, Kiyono M (2013b) Increase methylmercury accumulation in *Arabidopsis thaliana* expressing bacterial broad-spectrum mercury transporter MerE. AMB Express 3(1):52

Stoppel RD, Schlegel HG (1995) Nickel-resistant bacteria from anthropogenically nickel-polluted and naturally nickel-percolated ecosystems. Appl Environ Microbiol 61(6):2276–2285

Stoyanov JV, Brown NL (2003) The *Escherichia coli* copper-responsive copA promoter is activated by gold. J Biol Chem 278(3):1407–1410

Stoyanov JV, Hobman JL, Brown NL (2001) CueR (YbbI) of *Escherichia coli* is a MerR family regulator controlling expression of the copper exporter CopA. Mol Microbiol 39(2):502–511

Taghavi S, Delanghe H, Lodewyckx C, Mergeay M, van der Lelie D (2001) Nickel-resistance-based minitransposons: new tools for genetic manipulation of environmental bacteria. Appl Environ Microbiol 67(2):1015–1019

Taghavi S, Lesaulnier C, Monchy S, Wattiez R, Mergeay M, van der Lelie D (2009) Lead(II) resistance in *Cupriavidus metallidurans* CH34: interplay between plasmid and chromosomally-located functions. Anton Leeuw Int J G 96(2):171–182

Tibazarwa C, Corbisier P, Mench M, Bossus A, Solda P, Mergeay M, Wyns L, van der Lelie D (2001) A microbial biosensor to predict bioavailable nickel in soil and its transfer to plants. Environ Pollut 113(1):19–26

Tibazarwa C, Wuertz S, Mergeay M, Wyns L, van der Lelie D (2000) Regulation of the *cnr* cobalt and nickel resistance determinant of *Ralstonia eutropha* (*Alcaligenes eutrophus*) CH34. J Bacteriol 182(5):1399–1409

Top E, Desmet I, Verstraete W, Dijkmans R, Mergeay M (1994) Exogenous isolation of mobilizing plasmids from polluted soils and sludges. Appl Environ Microbiol 60(3):831–839

Top E, Mergeay M, Springael D, Verstraete W (1990) Gene escape model: transfer of heavy metal resistance genes from *Escherichia coli* to *Alcaligenes eutrophus* on agar plates and in soil samples. Appl Environ Microbiol 56(8):2471–2479

Top EM, Derore H, Collard JM, Gellens V, Slobodkina G, Verstraete W, Mergeay M (1995) Retromobilization of heavy-metal resistance genes in unpolluted and heavy-metal polluted soil. FEMS Microbiol Ecol 18(3):191–203

Tricot C, van Aelst S, Wattiez R, Mergeay M, Stalon V, Wouters J (2005) Overexpression, purification, crystallization and crystallographic analysis of CopK of *Cupriavidus metallidurans*. Acta Crystallogr, Sect F: Struct Biol Cryst Commun 61(Pt 9):825–827

Utschig LM, Bryson JW, O'Halloran TV (1995) Mercury-199 NMR of the metal receptor site in MerR and its protein-DNA complex. Science 268(5209):380–385

Valls M, Atrian S, de Lorenzo V, Fernandez LA (2000) Engineering a mouse metallothionein on the cell surface of *Ralstonia eutropha* CH34 for immobilization of heavy metals in soil. Nat Biotechnol 18(6):661–665

van Aelst S (2008) Functional study of plasmid-bourne *cop* genes of *Cupriavidus metallidurans* CH34: physiological, biochemical et ecological aspects. Université Libre de Bruxelles, Brussels, Belgium

Van der Auwera GA, Krol JE, Suzuki H, Foster B, Van Houdt R, Brown CJ, Mergeay M, Top EM (2009) Plasmids captured in *C. metallidurans* CH34: defining the PromA family of broad-host-range plasmids. Anton Leeuw Int J G 96(2):193–204

van der Lelie D, Schwuchow T, Schwidetzky U, Wuertz S, Baeyens W, Mergeay M, Nies DH (1997) Two-component regulatory system involved in transcriptional control of heavy-metal homoeostasis in *Alcaligenes eutrophus*. Mol Microbiol 23(3):493–503

van Elsas JD, Gardener BB, Wolters AC, Smit E (1998) Isolation, characterization, and transfer of cryptic gene-mobilizing plasmids in the wheat rhizosphere. Appl Environ Microbiol 64(3):880–889

Van Houdt R, Monchy S, Leys N, Mergeay M (2009) New mobile genetic elements in *Cupriavidus metallidurans* CH34, their possible roles and occurrence in other bacteria. Anton Leeuw Int J G 96:205–226

Van Houdt R, Monsieurs P, Mijnendonckx K, Provoost A, Janssen A, Mergeay M, Leys N (2012) Variation in genomic islands contribute to genome plasticity in *Cupriavidus metallidurans*. BMC Genom 13:111

Van Houdt R, Toussaint A, Ryan MP, Pembroke JT, Mergeay M, Adley CC (2013) The Tn*4371* ICE family of bacterial mobile genetic elements. In: Roberts AP, Mullany P (eds) Bacterial integrative mobile genetic elements. Landes Bioscience, Austin, pp 179–200

van der Lelie D, Corbisier P, Baeyens W, Wuertz S, Diels L, Mergeay M (1994) The use of biosensors for environmental monitoring. Res Microbiol 145(1):67–74

von Rozycki T, Nies DH (2009) *Cupriavidus metallidurans*: evolution of a metal-resistant bacterium. Anton Leeuw Int J G 96(2):115–139

Waldron KJ, Robinson NJ (2009) How do bacterial cells ensure that metalloproteins get the correct metal? Nat Rev Microbiol 7(1):25–35

Weyens N, Truyens S, Saenen E, Boulet J, Dupae J, Taghavi S, van der Lelie D, Carleer R, Vangronsveld J (2011) Endophytes and their potential to deal with co-contamination of organic contaminants (toluene) and toxic metals (nickel) during phytoremediation. Int J Phytorem 13(3):244–255

Wiesemann N, Mohr J, Grosse C, Herzberg M, Hause G, Reith F, Nies DH (2013) Influence of copper resistance determinants on gold transformation by *Cupriavidus metallidurans* Strain CH34. J Bacteriol 195(10):2298–2308

Wilson JR, Leang C, Morby AP, Hobman JL, Brown NL (2000) MerF is a mercury transport protein: different structures but a common mechanism for mercuric ion transporters? FEBS Lett 472(1):78–82

Yuan C, Lu X, Qin J, Rosen BP, Le XC (2008) Volatile arsenic species released from *Escherichia coli* expressing the As(III) S-adenosylmethionine methyltransferase gene. Environ Sci Technol 42(9):3201–3206

Zhang YB, Monchy S, Greenberg B, Mergeay M, Gang O, Taghavi S, van der Lelie D (2009) ArsR arsenic-resistance regulatory protein from *Cupriavidus metallidurans* CH34. Anton Leeuw Int J G 96(2):161–170

Zimmermann M, Udagedara SR, Sze CM, Ryan TM, Howlett GJ, Xiao Z, Wedd AG (2012) PcoE–a metal sponge expressed to the periplasm of copper resistance *Escherichia coli*. Implication of its function role in copper resistance. J Inorg Biochem 115:186–197

Zoropogui A, Gambarelli S, Covès J (2008) CzcE from *Cupriavidus metallidurans* CH34 is a copper-binding protein. Biochem Biophys Res Commun 365(4):735–739