

# Chapter 9

## Responses of Lactic Acid Bacteria to Heavy Metal Stress

Marc Solioz, Mélanie Mermod, Helge K. Abicht, and Stefano Mancini

### 9.1 Introduction

Lactic acid bacteria (LAB) belong to the order *Lactobacillales* and produce lactic acid as a result of carbohydrate fermentation. They are widely used in the production of fermented food, such as yogurt (streptococci and lactobacilli), cheeses (lactococci), sauerkraut (*Leuconostoc*), wine (oenococci), or cured sausages like Salami (pediococci, lactococci). They are heterotrophic and generally have complex nutritional requirements because they lack many biosynthetic capabilities. Because of this, LAB are generally abundant only in environments where these requirements can be provided, such as animal oral cavities and intestines (e.g., enterococci), plant leaves (*Lactobacillus*, *Leuconostoc*), decaying plant or animal matter, feces, and compost, etc.

LAB are used in the food industry for several reasons. Their growth lowers both the carbohydrate content of the foods that they ferment and the pH due to lactic acid production. This is often accompanied by the secretion of bacteriocins, such as nisin. Bacteriocins are proteinaceous toxins that inhibit the growth of similar or closely related bacterial strains. The combined action of low pH and bacteriocins is very important in food preservation to inhibit efficiently the growth of competing bacteria, including the most common human pathogens (Galvez et al. 2007). This bestows prolonged shelf lives on these foods. The acidity also changes the texture of the foods due to the precipitation of some proteins. In addition, the biochemical conversions involved in growth greatly enrich the flavor of fermented food. The acidic ambient generated by the secreted lactic acid can lead to the solubilization of complexed metal ions. For example, in traditional cheese making, the cells are challenged by copper released from the copper kettles (Kiermeier and Kyrein 1971).

---

M. Solioz (✉)

Department Clinical Research, University of Bern, Murtenstrasse 35, 3010 Berne, Switzerland  
e-mail: marc.solioz@ikp.unibe.ch

Though this process is important for flavor development (Steffen et al. 2009), it also puts stress on the bacteria.

A distinction should be made between metal ions that are required by LAB for certain enzyme functions and are thus vital and metals that are only toxic without a benefit for life. Of the trace metals known to function in biochemical processes, iron, zinc, and magnesium are probably used by all bacteria, whereas nickel, cobalt, selenium, and molybdenum are only used by some. No function for copper or selenium has been identified in any member of the *Lactobacillales*, and only a few organisms of this order have an apparent requirement for nickel or cobalt (Table 9.1).

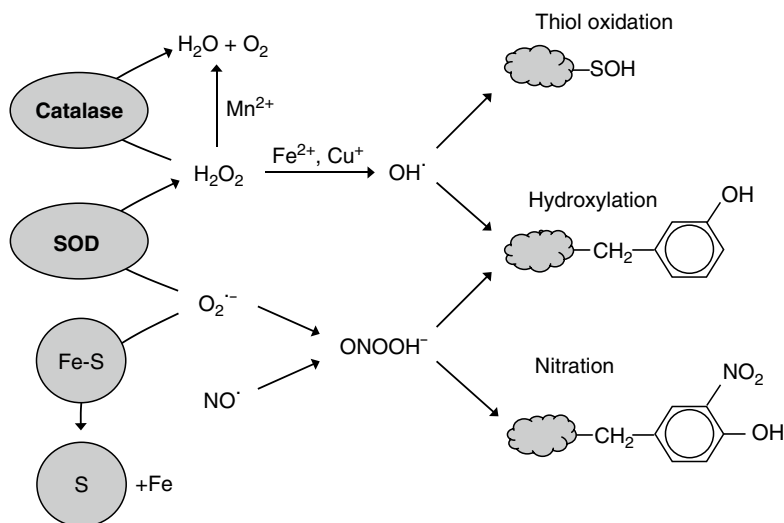
Some trace metals, like iron, selenium, molybdenum, manganese, and copper, are often scarce in the environment, and it can be assumed that cells requiring these metals possess corresponding uptake systems. However, few of these have been characterized to date. For essential metals like copper, nickel, cobalt, and zinc, which can occur in widely different bioavailable concentrations in the environment, bacteria must have homeostatic control mechanisms that can deal with excess as well as with deficiency. For purely toxic metals without a known function in biology, like lead, silver, or cadmium, specialized defense mechanisms have evolved in many bacterial species (a biological function for cadmium has been described in a marine diatom, but this may represent an exceptional case (Lane and Morel 2000)). Unfortunately, the knowledge of metal homeostasis and defense against metal stress by LAB is still very limited. Of all the biologically relevant metals, copper by far has received the widest attention. Copper homeostasis and the response to copper stress have been studied in detail in *Lactococcus lactis* and *Enterococcus hirae* and will be a major focus of this chapter. Stress responses to other metals, which have received little interest in LAB, will also be discussed for related bacteria such as *Bacillus subtilis*, to the extent that such work could be relevant to LAB on the basis of the known gene complements. Vanadium, molybdenum, and tungsten, which serve as cofactors in a variety of bacterial enzymes, will not be discussed because they are generally rare in the environment and have not received any attention in LAB.

## 9.2 Metal Toxicity Mechanisms

Several reactive oxygen species (ROS) and one thiyl radical ( $RS^{\bullet}$ ) can be formed in cells and can exert toxicity by modifying biomolecules (see Miyoshi et al. (2003) for review). Metal ions can catalyze some of the reactions that lead to their formation, which is one of the underlying mechanisms of metal-induced stress. Superoxide radicals ( $O_2^{\bullet-}$ ) are formed when oxygen takes up one electron. It is a product of “leaks” in the mitochondrial electron transport chain, but it can also be produced by macrophages in the “oxidative burst,” which is an important bactericidal action by these cells.  $O_2^{\bullet-}$  can be directly toxic, for example, by oxidizing and displacing iron from Fe-S clusters (Fig. 9.1), whereby the released iron can catalyze additional toxic reactions (see ahead). Alternatively, superoxide can be converted by SOD to

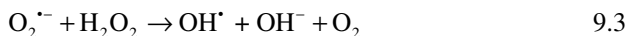
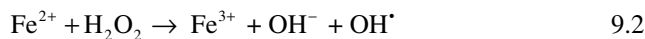
**Table 9.1** Use of selected metals by lactic acid bacteria

Species	Genomes	Cu users (Ridge et al. 2008)	Ni users (Zhang et al. 2009)	Co users (Zhang et al. 2009)	Mo users (Zhang and Gladyshev 2008)	Se users (Zhang et al. 2008)
<i>Enterococci</i>	2	0	0	1	1	0
<i>Lactobacilli</i>	10	0	0	7	2	0
<i>Lactococci</i>	1	0	0	0	0	0
<i>Leuconostoc</i>	1	0	0	0	0	0
<i>Oenococcus</i>	1	0	0	0	0	0
<i>Pediococcus</i>	1	0	0	0	0	0
<i>Streptococcus</i>	6	0	1	2	0	0



**Fig. 9.1** Major oxidative damage mechanisms and their coupling to redox-active metals. Superoxide ( $\text{O}_2^{\bullet-}$ ) produced by physiological reactions can attack iron-sulfur centers of enzymes and cause loss of the iron from the reactive center. For detoxification, superoxide is converted by superoxide dismutase (SOD) to hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Hydrogen peroxide can be dismutated to water and oxygen either in a nonenzymatic reaction with  $\text{Mn}^{2+}$  or by catalase, but can also undergo Fenton chemistry catalyzed by  $\text{Fe}^{2+}$  or  $\text{Cu}^+$ , resulting in highly toxic hydroxyl radicals ( $\text{OH}^\bullet$ ). These can lead to thiol oxidation and hydroxylation of cellular constituents. Superoxide also can react with nitrous oxide radicals ( $\text{NO}^\bullet$ ) to form reactive peroxynitrite ( $\text{ONOOH}^-$ ), which can nitrate or hydroxylate cellular components

less toxic hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). However, the combination of iron,  $\text{H}_2\text{O}_2$ , and superoxide leads to the generation of hydroxyl radicals ( $\text{OH}^\bullet$ ) by a combination of the Fenton reaction (9.2) and the Haber–Weiss reaction (9.3):



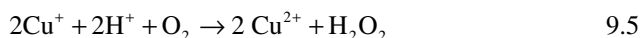
$\text{OH}^\bullet$  are very reactive and damage biomolecules by hydroxylation. In the overall ROS scheme, iron, copper, and other redox-active metal ions exert their effects by stimulating the Fenton reaction.

$\text{H}_2\text{O}_2$  is mainly produced by enzymatic reactions, such as the dehydrogenation of NAD(P)H. Because most lactococci do not possess catalase,  $\text{H}_2\text{O}_2$  can reach levels of 1–2% (Rochat et al. 2006) and may thus be considered not very toxic per se. It can leave the cells by diffusion through the membrane.  $\text{H}_2\text{O}_2$  is also produced by the

dismutation of  $O_2^{\cdot-}$  by superoxide dismutase (SOD). The combination of SOD and catalase provides an efficient antioxidant mechanism.

Nitric oxide is naturally formed in activated macrophages and endothelial cells and is considered as an active agent in several pathologies based on inflammation and organ reperfusion and may also play an important role in atherosclerosis. It has poor oxidizing power and is even antioxidant under physiological concentrations (up to 100 nM). It does, however, react rapidly with oxygen to yield nitrogen dioxide ( $NO_2^{\cdot}$ ), which in turn may react with  $NO^{\cdot}$  to yield nitrogen trioxide,  $N_2O_3$ . The rapid reaction of  $O_2^{\cdot-}$  with  $NO^{\cdot}$  gives the extremely reactive peroxyxynitrite,  $ONOO^-$ , which mediates oxidation, nitrozylation, and nitration reactions.

Aliphatic thiols, RSH, are contained in living organisms in high concentrations. Typical levels of intracellular glutathione are 5–10 mM, and a similar level of RSH is provided by cysteines in proteins. RSH can be oxidized in the presence of redox-active metal ions like iron or copper ions according to reactions (9.4) and (9.5):



The thiyl radicals have strong reactivity toward oxygen (9.6):



Furthermore, thiyl radicals are able to oxidize NADH to  $NAD^{\cdot}$ , as well as ascorbic acid, and to generate various free radicals such as  $OH^{\cdot}$  and  $O_2^{\cdot-}$ . There can also be thiol depletion by reaction (9.7) in cyclic combination with reaction (9.7):



While lipid and protein damage by the above mechanism has been demonstrated in vitro in many studies, recent findings suggest that alternative mechanisms of metal toxicity may be responsible for the primary toxic effects of copper, iron, and related metals in vivo. First, the discovery that free copper or iron in the cell is extremely low or even nonexistent makes Fenton chemistry and sulfhydryl depletion very unlikely mechanisms (Changela et al. 2003). Second, most *Lactobacillales* are rather tolerant to  $H_2O_2$ . For example *L. lactis* IL1403, described in some detail below, generates  $H_2O_2$  by NADH dehydrogenation but does not possess catalase for  $H_2O_2$  removal (Bolotin et al. 2001; Marty-Teyssset et al. 2000; Rochat et al. 2006). Third, Macomber et al. recently showed that copper-loaded *Escherichia coli* was less sensitive to killing by  $H_2O_2$  than cells grown without copper. Also, copper decreased the rate of  $H_2O_2$ -induced DNA damage. High intracellular copper levels even impaired iron-mediated oxidative killing by  $H_2O_2$  (Macomber et al. 2007).

Based on these observations, the authors suggested that copper exerts its toxicity by mechanisms other than oxidative stress.

A novel mechanism of copper toxicity was indeed recently demonstrated. It could be shown *in vivo* as well as *in vitro* that copper specifically damaged the iron-sulfur clusters of isopropylmalate dehydratase of *E. coli* (Macomber and Imlay 2009). This enzyme of the branched-chain amino acid biosynthesis pathway contains an iron-sulfur cluster from which the iron can be displaced by copper in the absence of oxygen. Copper efflux systems, chelation by glutathione, and cluster repair by assembly systems all enhance the resistance of cells to this type of copper toxicity. To establish whether this mechanism is a general route of copper toxicity in bacteria, including LAB, will require further investigation.

### 9.3 Response to Copper and Silver

#### 9.3.1 Copper as a Bioelement

In the primordial, anaerobic world, copper was in the Cu(I) state in the form of water-insoluble sulfides under neutral pH conditions and was only bioavailable in the acidic waters near hydrothermal vents. The emergence of an oxygen-containing atmosphere by the action of oxygen-evolving microorganisms, probably cyanobacteria, less than  $3 \times 10^9$  years ago was a dramatic event for most living organisms (Kasting and Siefert 2002). Most of them adapted to the new conditions by acquiring an oxidative metabolism. The “old” enzymes involved in anaerobic metabolism were designed to operate in the lower portion of the redox spectrum. The arrival of dioxygen created the need for a new redox active metal that could attain higher redox potentials. The oxidation of insoluble Cu(I) led to soluble and thus widely bioavailable Cu(II), which was ideally suited to exploit the oxidizing power of dioxygen (Crichton and Pierre 2001). Copper therefore is a modern bioelement (Kaim and Rall 1996). Concomitant with the arrival of oxygen, multicellular organisms developed.

Because of copper's ability to cycle between  $\text{Cu}^{2+}$  and  $\text{Cu}^+$  at biologically relevant redox potentials, it has become a cofactor for over 30 known enzymes in higher organisms (Karlin 1993). Prominent examples are lysyl oxidase, involved in the cross-linking of collagen; tyrosinase, required for melanin synthesis; dopamine  $\beta$ -hydroxylase of the catecholamine pathway; cytochrome *c* oxidase as a terminal electron acceptor of the respiratory chain; and SOD, required for defense against oxidative damage. Another class of copper proteins, such as plastocyanins or azurins, acts as electron carriers. Depending on the type of coordination of the copper to the protein, the redox potential can vary over the range of +200 to +800 mV. Concomitant with the lower complexity of bacteria, only ten cuproenzymes have so far been characterized in microbes (Table 9.2).

Strikingly, none of the sequenced *Lactobacillales* appears to be copper users based on bioinformatics analysis of known copper enzymes. The definition of

**Table 9.2** Known bacterial copper-containing enzymes

Enzyme	Function	References
Cytochrome <i>c</i> oxidase	Terminal oxidase	Cavet et al. (2003)
NADH dehydrogenase-2	Electron transport, Cu reduction	Rapisarda et al. (2002); Rodriguez-Montelongo et al. (2006)
Nitrosocyanin, cupredoxin-like	Electron transfer, other?	Arciero et al. (2002)
Plastocyanins	Electron transfer	Cavet et al. (2003)
Cu-containing nitrite reductases	Nitrite reduction	Ellis et al. (2007)
Tyrosinase	Phenol oxidation, melanin synthesis	Lopez-Serrano et al. (2004); Tsai and Lee (1998)
Cu amine oxidases	Oxidation of primary amines	Brazeau et al. (2004)
Particulate methane monoxygenase	Methane oxidation	Chan et al. (2004)
Cu,Zn-superoxide dismutase (cuprein)	Defense during infection?	Battistoni (2003)
Cu-containing laccase	Polyphenol oxidase	Hullo et al. (2001)

“users” is obviously based on the currently known bacterial cuproenzymes summarized in Table 9.2. However, not all functions of copper in LAB are known. It was observed, for example, that *Lactococcus lactis* subsp. *lactis* 3022 produced more biomass when grown aerobically with hemin and copper (Kaneko et al. 1990). The activity of diacetyl synthase was greatly stimulated by the addition of hemin or copper, and the activity of NAD-dependent diacetyl reductase was very high. Pyruvate formed via glycolysis was converted to diacetyl, which in turn was converted to acetoin by the NAD-dependent diacetyl reductase to reoxidize NADH. This suggests that hemin or copper stimulates acetyl coenzyme A formation from pyruvate, but the nature of this mechanism remains unknown. At any rate, some bacteria make extensive use of copper as a bioelement, while others, like the *Lactobacillales*, use it for only a few functions, if at all. It might be speculated that there is a connection between the small average genome size of *Lactobacillales* of only 2.3 Mb and those of copper-using Gram-positive organisms, with an average genome size of 3 Mb (Ridge et al. 2008).

Recently, an unexpected link between copper and molybdenum cofactor (MOCO) synthesis was discovered. Plant Cnx1G, a domain of the Cnx1GE protein, catalyzes the adenylation of molybdopterin. Cnx1G-bound molybdopterin was found to have copper bound to the molybdopterin dithiolate sulfurs (Kuper et al. 2004). The function of this bound copper is presently unknown, but copper might play a role in protecting the molybdopterin dithiolate from oxidation and/or in presenting a suitable leaving group for molybdenum insertion (Schwarz and Mendel 2006). It remains currently unclear if the binding of copper to molybdopterin is an essential step in MOCO synthesis, but if so, this pathway generates a copper requirement in addition to those considered in Table 9.1 (Zhang and Gladyshev 2008). If one looks across the bacterial phyla, a cooccurrence of copper

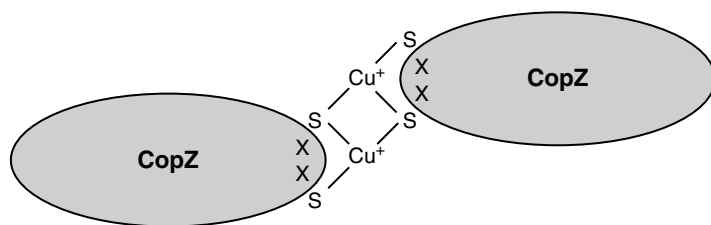


### 9.3.2.1 The CopZ Copper Chaperone

The identification of copper chaperones marked the emergence of a new concept in the handling of metal ions by cells, namely, the escorting of the metal by a protein to prevent nonspecific, damaging interactions. There is a range of different copper chaperones in eukaryotes to deliver copper to cytochrome *c* oxidase, SOD, or copper ATPases (Kim et al. 2008), but only two types of copper chaperones have so far been described in bacteria. Sco-like chaperones deliver copper to cytochrome oxidase; they appear to be absent in LAB. CopZ-like chaperones, on the other hand, transport copper to ATPases and transcriptional regulators and are ubiquitous in LAB (Huffman and O'Halloran 2001). Interestingly, no CopZ-like copper chaperones have been described in *Actinobacteria* or in *E. coli* and related organisms and it remains unknown how copper is escorted in the cytoplasm of those organisms.

CopZ of *E. hirae* is an 8-kDa protein, and the structure of CopZ and that of other CopZ-like proteins have been solved (see Davis and O'Halloran (2008) for review). They all share the same  $\beta\alpha\beta\beta\alpha\beta$  ferredoxin-like structure, with two exposed cysteines of a CxxC motif located in a loop between  $\beta 1$  and  $\alpha 1$ . There is still uncertainty as to how  $\text{Cu}^+$  is complexed by the chaperone in vivo. In principle,  $\text{Cu}^+$  can bind to the CxxC motif in a near-linear S–Cu–S bonding. However, X-ray structures of Hah1, the human CopZ-like copper chaperone, have revealed structures where a single  $\text{Hg}^{2+}$  or a  $\text{Cu}^+$  ion is complexed by the four cysteines of two chaperones in a dimeric arrangement (Rosenzweig 2001).  $\text{Cu}^+$ –CopZ of *E. hirae*, on the other hand, appeared to be dimeric in solution, with triagonally bound copper to be the most likely structure (Fig. 9.3) (Wimmer et al. 1999). The prevalence of homodimeric  $\text{Cu}^+$ –CopZ was also demonstrated by biochemical and light-spectroscopic techniques (Kihlken et al. 2002, 2008). A three-coordinate metal center is also supported by EXAFS measurements of  $\text{Cu}^+$ –thiol bonds (Pufahl et al. 1997; Wimmer et al. 1999). Glutathione was shown to inhibit dimer formation in vitro and could, in principle, be a ligand to monomeric  $\text{Cu}^+$ –CopZ inside the cell, where glutathione concentrations are high. It is also conceivable that there is an equilibrium among monomeric, dimeric, and even trimeric CopZ in the cell, but this will be difficult to assess.

CopZ of *E. hirae* was shown by surface plasmon resonance to interact with the CopA copper ATPase and the CopY repressor (Multhaup et al. 2001; Portmann et al. 2004). It is assumed that  $\text{Cu}^+$  imported by CopA is transferred to the CopZ



**Fig. 9.3** Model of  $\text{Cu}^+$ –CopZ dimer formation. Each  $\text{Cu}^+$  ion is coordinated by three sulfur atoms of the cysteine ligands of two CopZ molecules

copper chaperone, which subsequently delivers copper to the CopY repressor for induction of the *cop* operon (discussed ahead) or to other sites requiring copper. An interaction of CopZ with the CopB copper-exporting ATPase has also been shown (unpubl. observation), suggesting that CopZ, in addition, has a role in copper export from the cell. CopZ interaction with the copper-exporting ATPase was also demonstrated in *B. subtilis* (Radford et al. 2003). In eukaryotes, the primary function of CopZ-like copper chaperones (Hah1, Atx1, Atox1) is in fact the delivery of copper to copper ATPases (Huffman and O'Halloran 2001).

### 9.3.2.2 Copper ATPases

The two *E. hirae* copper ATPases mark the discovery of ATP-driven copper transport across cell membranes in 1992 (Odermatt et al. 1992). Before that time, there was no concept and no serious discussion of how copper could cross cell membranes. According to the current model, CopA serves in the uptake of copper when copper is limiting, while CopB serves in copper extrusion under conditions of copper excess (Odermatt et al. 1994; Solioz and Odermatt 1995) (the nomenclature is confusing: Copper export is accomplished by CopB in *E. hirae*, but by enzymes called “CopA” in most other bacteria).

Copper ATPases belong to the superfamily of P-type ATPases, classically represented by eukaryotic Ca- and NaK-ATPases. The most prominent feature of this family of pumps is the formation of an acylphosphate intermediate (hence the name P-type ATPases) whereby the  $\gamma$ -phosphate of ATP phosphorylates the aspartic acid residue in the conserved motif DKTGT during the reaction cycle (Pedersen and Carafoli 1987). Detailed structures of the calcium ATPase of the sarcoplasmic reticulum have given considerable insight into the working of such ATP-driven ion pumps (Toyoshima et al. 2003; Toyoshima and Mizutani 2004). Copper-transporting ATPases are a subgroup of the P-type ATPases. They have been termed heavy metal ATPases, or CPx-type ATPases due to a conserved intramembranous CPC or CPH motif (Lutsenko and Kaplan 1995; Solioz and Vulpe 1996), or P1B-type ATPases, based on more systematic phylogeny (Lutsenko and Kaplan 1995). CPx-type ATPases are widespread in nature and have been found to catalyze the transport of a range of transition and heavy metal ions, including  $\text{Cu}^+$ ,  $\text{Ag}^+$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$ , and  $\text{Pb}^{2+}$  (Axelsen and Palmgren 1998).

Another typical feature of CPx-type ATPases is the presence of N-terminal metal-binding domains. These domains can be of two kinds. In most CPx-type ATPases, including CopA of *E. hirae*, these domains consist of a CopZ-like module with a conserved CxxC motif for copper binding and the same  $\beta\alpha\beta\beta\alpha\beta$  fold exhibited by CopZ-like chaperones. Some prokaryotic ATPases possess two such CopZ-like domains, and eukaryotic copper ATPases have two (yeast) or six (humans) such domains (Hanson et al. 2001; Solioz et al. 1994). The second type of N-terminal metal-binding domain is found in CopB of *E. hirae* and a few poorly characterized bacterial CPx-type ATPases. It consists of a histidine- and methionine-rich region. Similar repeat structures were also found in two *Pseudomonas syringae* proteins,

which were demonstrated to be periplasmic copper-binding proteins (Cha and Cooksey 1991).

The function of the N-terminal metal-binding domains of heavy metal ATPases remains unclear. Copper transfer from chaperones to the N-terminal metal-binding domains of CPx-type ATPases is now well documented, but it has never been shown that this copper can actually be transported across the membrane. Rather, it has been suggested that the N-terminus regulates the activity of the ATPase by domain-domain interaction (Arguello and Gonzalez-Guerrero 2008). Copper transport may thus require a separate copper-donation event by the chaperone to the membrane region of the ATPases (Gonzalez-Guerrero and Arguello 2008). In *B. subtilis*, the copper export pump CopA features two N-terminal CopZ-like copper-binding domains. It was shown that these motifs play a role in the dimerization of CopA, which could constitute a regulatory mechanism of the ATPase (Singleton et al. 2008; Singleton and Le Brun 2009).

ATP-driven copper transport from the cytoplasm to the extracytoplasmic space, catalyzed by copper ATPases, has been extensively studied and appears to take place in all bacterial species. Both  $\text{Cu}^+$  and  $\text{Ag}^+$  export by CopB of *E. hirae* have been directly demonstrated with radioisotopes in membrane vesicle and in whole cells loaded with silver (Odermatt et al. 1994; Solioz and Odermatt 1995). Copper-importing ATPases, on the other hand, have only been described in *E. hirae* (CopA), *Synechocystis* sp. (CtaA), and *B. subtilis* (YcnJ) (Chillappagari et al. 2009; Odermatt et al. 1994; Tottey et al. 2001). While the role of *E. hirae* CopA in cell physiology is still unclear, the CtaA of *Synechocystis* sp. imports copper for plastocyanin, a copper-containing thylakoid protein that functions in the photosynthetic electron transport chain.

Cyanobacteria (e.g., *Synechocystis* sp.) are the one bacterial group that has a known demand for cytoplasmic copper for the synthesis of copper-containing, thylakoid-localized plastocyanin and cytochrome oxidase (Tottey et al. 2005). In other organisms, the cuproenzymes are localized at the cytoplasmic membrane or in the periplasm, and copper loading of these proteins could take place in the periplasmic space. In many bacteria, including LAB, no intracellular copper requirements are known at all. The copper homeostatic machinery of these organisms may thus have the sole purpose of keeping copper out. Nevertheless, specific copper importers that are expressed under copper-limiting conditions have been described in *E. hirae* and *B. subtilis* (Chillappagari et al. 2009; Wunderli-Ye and Solioz 2001). Energy-dependent copper uptake has, however, not been directly demonstrated, neither by copper ATPases nor by alternative mechanisms such as with chalkophores (copper “siderophores”) (Balasubramanian and Rosenzweig 2008; Kim et al. 2004) or as copper-substrate complexes through substrate transporters. In this light, copper import into the cytoplasm of LAB still needs rigorous experimental confirmation.

### 9.3.2.3 Regulation of Copper Homeostatic Genes

CopY of *E. hirae* is a copper-responsive transcriptional regulator. It responds to excessive copper in the cytoplasm by derepressing the *cop* operon. In LAB, two

**Table 9.3** Copper-responsive regulators of *Actinobacteria*, *Firmicutes*, and *Proteobacteria*

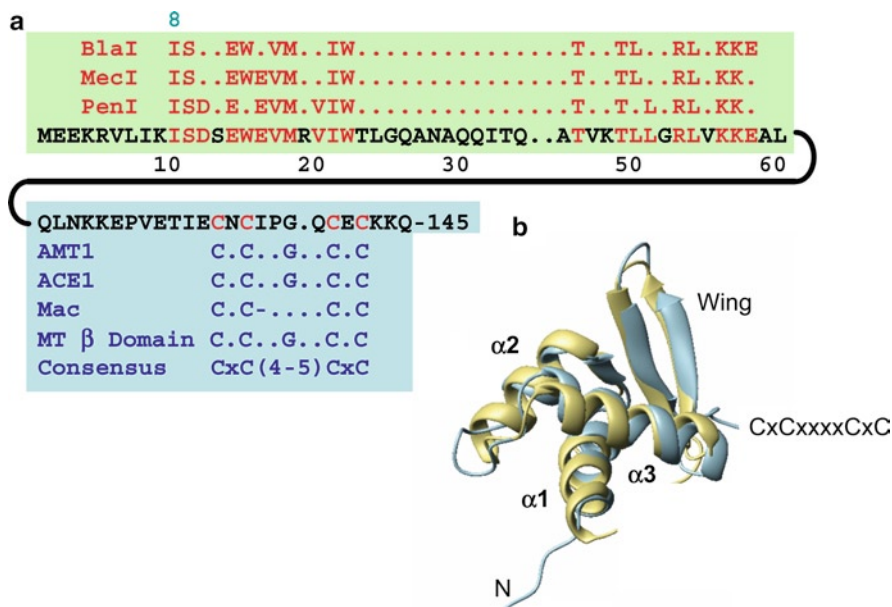
Organisms	CopY-type	CsoR-type	CueR-type
<i>Actinobacteria</i>	0	43	0
<i>Firmicutes</i>			
Bacillales	3	30	7
Clostridia	0	25	0
Lactobacillales	39	3	0
<i>Proteobacteria</i>	0	54	215

types of copper-responsive transcriptional regulators have been identified by bioinformatics analysis of sequenced genomes, namely, CopY-type and CsoR-type regulators (Table 9.3). CopY-like repressors are the principal copper-responsive regulators of LAB and have experimentally been associated with gene regulation in *E. hirae* (Strausak and Solioz 1997), *Enterococcus faecium* (Hasman et al. 2006), *L. lactis* IL1403 (Magnani et al. 2008), *Streptococcus mutans* (Portmann et al. 2006; Vats and Lee 2001), and *Streptococcus gordonii* (Mittrakul et al. 2004).

CsoR-type regulators have only recently been described (Liu et al. 2007), although their occurrence is more widespread in the prokaryotic world than that of CopY-type repressors. In LAB, CsoR-related proteins occur only in a minority of the sequenced species, and no biochemical studies are as yet available. Finally, CueR-type regulators, which regulate copper homeostatic genes in *E. coli* (Outten et al. 2000), only occur in a few species of the *Bacillales*, but not in LAB. CueR-type regulators are thus the primary copper-responsive regulators of Gram-negative bacteria.

CopY has a bipartite structure: The N-terminus interacts with DNA, while the C-terminus interacts with zinc or copper. The N-terminus shows extensive sequence similarity to BlaI, MecI, and PenI, repressors that are involved in the regulation of  $\beta$ -lactamase in Gram-positive bacteria (Fig. 9.4a) (Garcia-Castellanos et al. 2004; Himeno et al. 1986; Van Melckebeke et al. 2003; Wittman and Wong 1988). The structure of the N-terminus of CopR of *L. lactis*, a CopY-homolog, has been solved by solution NMR (Cantini et al. 2009) and is in fact nearly superimposable on the structure of BlaI of *Bacillus licheniformis* (Fig. 9.4b). The C-terminus of CopY exhibits sequence similarity to the yeast copper-inducible repressors AMT1, ACE1, and Mac, and to the  $\beta$ -domain of metallothioneins (Bird 2008). All these proteins feature the consensus motif CxCX<sub>4-5</sub>CxC. In newly synthesized CopY, this site is occupied by a single Zn<sup>2+</sup>, which is coordinated by four sulfur atoms in a tetrahedral fashion (Cobine et al. 2002b).

At low ambient copper concentrations, CopY is present as a Zn(II)-containing homodimer and is bound to the operator-promoter region of the operon (Strausak and Solioz 1997). The CopY-dimer-binding sites feature the so called *cop*-box of consensus TACAnnTGTA, a motif that is widely conserved in the *Lactobacillales*. The DNA–CopY interaction has been assessed in quantitative terms by surface plasmon resonance analysis (Portmann et al. 2006). It was found that the CopY-type repressors of *L. lactis*, *E. hirae*, or *S. mutans* had very similar affinities for *cop*-boxes (Portmann et al. 2004). Interestingly, the  $\beta$ -lactamase regulators BlaI, MecI,



**Fig. 9.4** (a) Alignment of the protein sequence of CopY of *E. hirae* with those of  $\beta$ -lactamase regulators in the N-terminal region and fungal transcriptional regulators and metallothionein in the C-terminal region. (b) Overlay of the N-terminal DNA-binding domain of *L. lactis* CopR (blue) and the BlaI  $\beta$ -lactamase regulator of *B. licheniformis* (gold)

and PenI, which feature an N-terminal DNA-binding domain essentially identical to that of CopY-like repressors, also recognize a “cop-box” (Sharma et al. 1998); the possible consequences of this have not been investigated.

Under low-copper conditions, a CopY dimer is bound to the cop-box and prevents transcription. When media copper is raised, two  $\text{Cu}^+$ -CopZ donate the copper ion to one CopY monomer. This displaces the bound  $\text{Zn(II)}$ , and CopY is released from the DNA as  $\text{Cu}_2^+$ -CopY, allowing transcription to proceed (Fig. 9.2). Protein–protein interaction between CopZ and CopY could be demonstrated by surface plasmon resonance spectroscopy (Multhaup et al. 2001), and the overall induction mechanism of CopY by copper and CopZ is experimentally well supported (Cobine et al. 1999, 2002a–c). At high intracellular copper levels, CopZ is degraded through a proteolytic pathway, conceivably because high levels of  $\text{Cu}^+$ -CopZ may be toxic to the cell (Lu and Solioz 2001). Following release from the DNA,  $\text{Cu}_2^+$ -CopY is probably also proteolytically degraded (unpubl. observation).

CsoR-type repressors have so far only been studied in *Mycobacterium tuberculosis* and *B. subtilis*. However, it can be assumed that CsoR-type repressors work similarly in LAB and will thus be briefly discussed. CsoR from *M. tuberculosis* represents the founding member of this new class of prokaryotic Cu(I) regulators, and its structure has recently been solved (Liu et al. 2007). CsoR is tetrameric, with two monomers each forming a stable homodimer that adopts an antiparallel

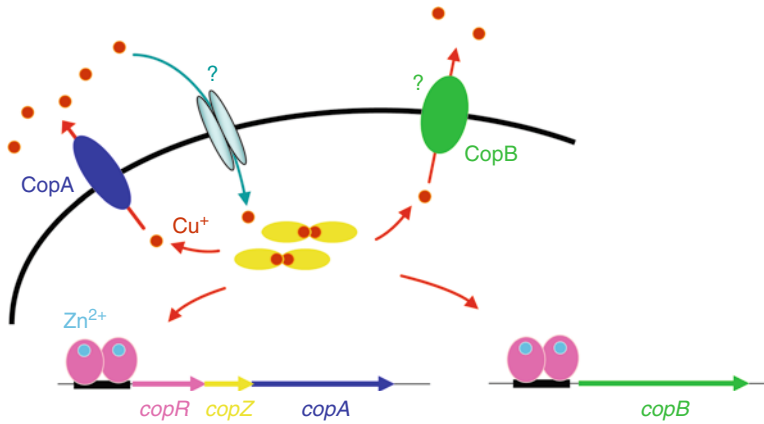
four-helix bundle architecture. This represents a novel DNA-binding fold because it lacks the obvious candidate DNA-binding domains present in winged-helix-type metalloregulators like CopY and CueR. Each CsoR homodimer binds two Cu<sup>+</sup> such that they bridge the two subunits. By X-ray absorption spectroscopy, it was shown that Cu<sup>+</sup> adopts a planar trigonal coordination involving two cysteines and a histidine residue (Liu et al. 2007).

CsoR has been shown to regulate the *copZA* operon of *B. subtilis* by copper-dependent derepression (Ma et al. 2009). The operon encodes a CopZ-type copper chaperone and a copper efflux ATPase. Two tetramers of apo-CsoR were shown to bind to a 30-bp DNA region overlapping the promoter of the *copAZ* operon. Cu<sup>+</sup> weakened the CsoR-DNA interaction, thereby inducing the operon (Liu et al. 2007). CopY- and CsoR-type repressors feature very different structures and activation mechanisms to fulfill essentially the same role. Why such diverse mechanisms for gene regulation by copper evolved remains an interesting open question.

In the study of the acid adaptation of *Lactobacillus bulgaricus*, it was found that, among a range of three dozen other genes, three CPx-type ATPases were induced by low-pH stress (Penaud et al. 2006). One of these ATPases resembles CopB of *E. hirae*, and it appears likely that it serves in copper extrusion. Acidic conditions can lead to an increase in ambient copper concentrations through the release of bound copper and the induction of copper-exporting ATPases by acid stress makes physiological sense. No *cop*-boxes were present in any of the promoters of the *L. bulgaricus* CPx-type ATPases and the induction mechanism by low pH remains unknown. Acid-sensitive mutants in the unrelated microorganisms *Rhizobium leguminosarum* bv. *viciae* and *Sinorhizobium meliloti* were similarly found to have disrupted *actP* genes that encode CPx-type ATPases (Reeve et al. 2002). These mutant strains were also more sensitive to ambient copper. Copper induced the expression of the wild-type *actP* genes and low pH enhanced the induction two to threefold. Downstream of the ATPase genes of both organisms are genes encoding MerR-type transcriptional regulators, termed HmrR, which apparently regulate the expression of the ActP ATPases. In *E. coli*, a MerR-type transcriptional regulator, CueR, is responsible for the copper-induced transcription of the CopA copper-exporting ATPase and the periplasmic CueO copper oxidase (Outten et al. 2000, 2001). The acid induction of copper ATPases may be a more general phenomenon. Unfortunately, the acid induction of the copper homeostatic genes of *E. coli*, *E. hirae*, and *L. lactis* has not been addressed so far.

### 9.3.3 Copper Homeostasis in *Lactococcus lactis*

In *L. lactis* IL1403, the copper-inducible *copRZA* operon encodes the CopR repressor, a CopY-type repressor, the CopZ copper chaperone, and the CopA copper ATPase (Fig. 9.5). The latter exhibits 45% sequence identity to CopA of *E. hirae*. In contrast to *E. hirae* CopA, *L. lactis* CopA has been shown to be a copper-exporting ATPase (Magnani et al. 2008). The CopR repressor of *L. lactis* regulates the CopR



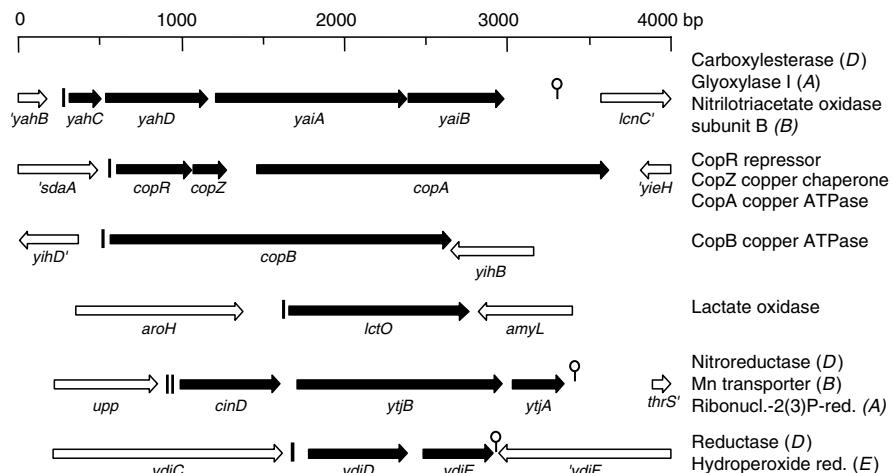
**Fig. 9.5** Illustration of copper homeostasis in *L. lactis*. How copper enters the cell is unknown. Excess cytoplasmic copper binds to CopZ, which can then donate  $\text{Cu}^+$  to either the copper ATPases for export or the CopR repressor to induce transcription. In low-copper conditions, a CopR dimer in the zinc form is bound to the *cop*-box in front of the *copRZA* operon and the *copB* gene. When CopZ donates  $\text{Cu}^+$  to CopR, one  $\text{Zn}^{2+}$  per CopR monomer is replaced by two  $\text{Cu}^+$ , with the concomitant release of CopR from the promoter and induction of transcription of the downstream genes. CopA then accomplishes copper export from the cytoplasm. The function of CopB is unknown

regulon in a fashion analogous to CopY of *E. hirae*. The CopZ-like copper chaperone, finally, can be assumed to function in intracellular copper routing (Aarnesano et al. 2002; Cobine et al. 2002b).

A second putative copper ATPase in *L. lactis* is encoded by the unlinked, monocistronic *copB* gene, which is also under the control of CopR. CopB features a histidine-rich N-terminus and shares 55% sequence identity with *E. hirae* CopB. However, no function could so far be assigned to this enzyme. It is notable that *E. hirae* CopB is encoded by the *copYZAB* operon, while CopB of *L. lactis* is encoded by a monocistronic gene. Whether these different gene organizations in *L. lactis* and *E. hirae* are a consequence of functional differences remains an open question.

### 9.3.3.1 Global Responses to Copper Stress by *Lactococcus lactis*

The CopR repressors of *L. lactis* also recognize the TACAnnTGTA consensus motif, the *cop*-box (Portmann et al. 2006). By performing a genome-wide search for *cop*-boxes in *L. lactis* IL1403, Barré and coworkers found 28 genes whose operator regions harbor the *cop*-box. Seven of these *cop*-boxes were shown to interact with CopR in a copper-responsive manner in vitro. The genes and operons associated with these *cop*-boxes were collectively termed *CopR regulon*. It encompasses a total of 14 genes, organized into four operons and two monocistronic genes (Fig. 9.6) (Magnani et al. 2008). What is the function of these additional copper-regulated genes in copper homeostasis? Only for *lctO*, an NAD-independent, flavin-containing lactate oxidase, has a function been proposed (Barré et al. 2007). Since LctO requires



**Fig. 9.6** CopR regulon of *L. lactis* IL1403. The genes in full color are regulated by CopR. Vertical lines indicate the location of *cop*-boxes and the *lariats* those of p-independent transcriptional terminators. The predicted functions of the genes are indicated on the right. Genes are drawn to the scale indicated in base pairs (bp) along the top of the figure

oxygen to convert lactate to pyruvate, it could serve in the elimination of molecular oxygen under copper stress, thereby attenuating the formation of reactive oxygen radicals (Barré et al. 2007). Similarly, an oxygen-consuming NADH oxidase has been proposed to be involved in the defense against oxidative stress in *Lactobacillus delbrueckii* subsp. *bulgaricus* by removing oxygen and thereby preventing the generation of  $H_2O_2$  and its reaction products (Marty-Teyssset et al. 2000).

### 9.3.4 Response to Silver

Silver has no known biological role and is highly toxic to microorganisms. In fact, silver-impregnated materials are starting to be employed to create aseptic surfaces or odorless clothing (Sondi and Salopek-Sondi 2004). Silver is not redox active like other toxic metals but remains in the  $Ag^+$  form. Silver has a very high affinity to thiolates and binds avidly to sites normally occupied by Cu(I). Intracellular copper is always in the form of Cu(I), due to the reducing environment of the cytoplasm, and any site normally occupied by copper can be taken over by silver. In this way, silver can activate the copper-responsive repressors like CopY of *E. hirae* or CopR of *L. lactis*, which has experimentally been verified (Odermatt and Solioz 1995). It has also been shown that silver can be a substrate for copper-transporting ATPases of bacteria, fish, and mammals (Bury et al. 1999; Hanson et al. 2001; Kanamaru et al. 1994; Stoyanov et al. 2003). The copper efflux ATPase of *E. hirae*, CopB, was shown to pump  $Ag^+$  with the same affinity and velocity as  $Cu^+$  (Solioz and Odermatt 1995).  $Ag^+$  also binds to copper chaperones in a fashion analogous to that of copper

(Kihlken et al. 2008; Narindrasorasak et al. 2004). Silver is thus a Cu(I) mimetic, and it can be assumed that all copper-resistance systems can also handle silver. However, due to the higher toxicity of silver, organisms can generally tolerate much less silver than copper.

A plasmid-born silver-resistance system has been isolated from silver-resistant *Salmonella* sp. (Gupta et al. 1999). The resistance determinant encodes a periplasmic silver-specific-binding protein plus two apparently parallel efflux pumps: a CPx-type ATPase, SilP, and a membrane potential-dependent cation/proton antiporter. The *sil* determinants are regulated by a two-component sensor kinase-response regulator system. Due to the similarity of  $\text{Ag}^+$  and  $\text{Cu}^+$ , it would be expected that the Sil system can also handle copper, but this was apparently not tested.

## 9.4 Response to Other Heavy Metals

Relatively few studies have been conducted on the response of LAB to heavy metals other than copper. We will therefore also discuss some of the key findings made in other bacterial species to the extent that they could be relevant to LAB. Mercury resistance, which has received considerable attention in many bacterial species, has not been addressed to any significant extent in LAB. The interested reader is referred to the excellent review on bacterial mercury resistance by Barkay et al. (2003).

### 9.4.1 Response to Iron

In air,  $\text{Fe}^{2+}$  is rapidly oxidized to  $\text{Fe}^{3+}$ , which forms hydroxides that are barely soluble at neutral pH. For this reason, bacteria generally have to deal with iron limitation rather than with iron excess. Hence, bacteria have developed a range of strategies to acquire iron from the environment. For one, they produce high-affinity chelators (siderophores) that can solubilize  $\text{Fe}^{3+}$ . In turn, corresponding ferrisiderophore-uptake systems take up the iron-siderophore complexes to cover the cellular demand for iron (Neilands 1995). It has been proposed that lactobacilli do not require iron for growth, based on the growth in iron-deficient media and other observations (Imbert and Blondeau 1998; Weinberg 1997). However, the genomes of LAB do contain genes that are predicted to have roles in iron acquisition. Also, recent work in this laboratory has identified HemN as an iron-requiring protein involved in heme metabolism. Conceivably, the iron requirement of LAB is conditional: Iron may only be required for aerobic growth, which also requires a supply of exogenous heme (Brooijmans et al. 2007).

LAB grown in the presence of oxygen produce damaging ROS, such as  $\text{H}_2\text{O}_2$ ,  $\text{OH}^\cdot$ , or  $\text{O}_2^{\cdot-}$ . The hydroxyl and superoxide radicals, rather than  $\text{H}_2\text{O}_2$ , represent the ROS causing toxicity for LAB.  $\text{H}_2\text{O}_2$  is membrane-permeable and can be accumulated in significant amounts by LAB. Many species, including *L. lactis* IL1403, do

not possess catalase for the removal of  $H_2O_2$  (Marty-Teyssset et al. 2000; Rochat et al. 2006). However, in the presence of iron,  $H_2O_2$  can be converted to highly reactive  $OH^\cdot$  by a Fenton-type reaction. Therefore, intracellular iron levels may contribute significantly to the impact of high- $H_2O_2$  levels on cell survival.

Since the importance of iron for the growth of LAB has been discounted, little work has been performed on iron homeostasis. Here, findings from related Gram-positive bacteria, such as *B. subtilis* or *Lactobacillus plantarum*, will also be discussed since they may be extrapolated to LAB and may serve as a starting point for further investigations. In *B. subtilis*, iron homeostasis has been investigated in some detail. In these cells, the ferric uptake regulator (Fur) represses genes involved in iron uptake. Fur is a dimeric DNA-binding protein with one structural  $Zn^{2+}$  ion per monomer and possesses a regulatory  $Fe^{2+}$ -binding site (Bsat and Helmann 1999; Kehres et al. 2000). Iron starvation induced by the treatment of cultures with the iron chelator 2,2'-dipyridyl induces the Fur regulon, encompassing 20 operons with 39 genes. The same set of genes is also induced in *fur*-deletion mutants, supporting the nature of the Fur regulon (Baichoo et al. 2002). *L. lactis* IL1403 possesses a Fur-like protein of similar size to *B. subtilis* Fur (128 vs. 132 amino acids) and with 28% sequence identity, but experimental evidence for a function of this protein in iron homeostasis is not available.

The analysis of Fur-regulated genes in *B. subtilis* has led to the identification of various iron-uptake pathways that may also be present in *L. lactis*, such as FeuB (Accession: ABX75613, 328 amino acids, 38% sequence identity to *B. subtilis* FeuB, 334 amino acids). In *B. subtilis*, there is a range of iron-uptake systems: FeuBC is believed to take up the siderophores enterobactin and corynebactin, the latter being the siderophore produced by *B. subtilis*. Except for the YebLMN elemental iron-uptake system (related to the yeast FTS3 system), the iron transporters identified in *B. subtilis* belong to the ABC transporter family (Andrews et al. 2003; Moore and Helmann 2005). Four ABC transporters for the uptake of ferric citrate, corynebactin, and hydroxamate-type siderophores appear to be present in *B. subtilis* (FeuBC, YfiZ/YfhA, FhuBG, and YfmDE). Of all these iron acquisition proteins, *L. lactis* appears to possess only FeuB. This suggests, on the one hand, that there is a need for iron uptake by this LAB but, on the other hand, indicates a very low, maybe even nonessential, demand for iron.

An ABC transporter, MtsABC, involved in iron and zinc uptake has also been described for *Streptococcus pyogenes* (Janulczyk et al. 1999). The isolated protein exhibited high-affinity binding of  $Zn(II)$ ,  $Fe(III)$ , and  $Cu(II)$  in vitro. An *mtsABC* mutant showed lower iron and zinc uptake but was not affected in its growth. In the light of these observations and of what is known about copper homeostasis, it appears unlikely that MtsABC acts as a copper importer in *S. pyogenes*. Convincing evidence of an ABC-type copper importer in any prokaryote has yet to be produced. All sequenced LAB genomes encode two or more ABC-type transporters, but the function of most of these has not yet been experimentally addressed.

Recently, an iron homeostatic gene, *mntH*, was identified in *Lactococcus lactis* MG1363, based on the resistance of tellurite ( $TeO_3^{2-}$ ) and oxidative stress (Turner et al. 2007). Tellurite exerts oxidative stress by superoxide formation, which

accompanies its reduction in the cytoplasm (Perez et al. 2007). The tellurite-resistant strain with a nonfunctional *mntH* gene exhibited greatly increased survival after 24 h of aerated growth, compared to the wild type. MntH is a member of the family of natural resistance-associated macrophage proteins (Nramp) (Richer et al. 2003). Members of this family have been shown to serve in  $Mn^{2+}$  and  $Fe^{2+}$  uptake (Kehres et al. 2000; Makui et al. 2000). The *mntH* mutant strain exhibited reduced iron uptake, suggesting that MntH serves in  $Fe^{2+}$  uptake. This observation does not, however, rule out that MntH also has a role in manganese acquisition (Turner et al. 2007). A strain deleted in MntH was still respiration-competent when supplied with heme or protoporphyrin IX, indicating that iron is still taken up. However, excess iron may be taken up by the wild type via MntH, and this iron could participate in oxygen-dependent toxicity in *L. lactis*.

### 9.4.2 Response to Zinc

Zinc is an essential metal ion but can be toxic if in excess. It plays a vital role as a cofactor for more than 300 enzymes, such as SOD, alcohol dehydrogenase, and DNA-binding proteins. It also functions as a structural scaffold for RNA polymerase, tRNA synthases, and approximately 40 additional proteins (Coleman 1998; Dunn et al. 2003; Outten and O'Halloran 2001; Sun and Plapp 1992; Vallee and Falchuk 1993). Additionally, zinc can also function as an antioxidant by protecting sulfhydryl groups of proteins from the attack of reactive free radical species and by antagonizing free radical formation by competing with redox-active transition metals like copper and iron (Powell 2000). In line with this, a mutant of *L. lactis* deficient in the low- and high-affinity zinc-uptake system was found to be more sensitive to  $H_2O_2$  (Scott et al. 2000). In *B. subtilis*,  $H_2O_2$  induces the Fur-like PerR repressor, which controls the expression of a dedicated zinc-uptake system, ZosA, in addition to catalase and some other genes (Gaballa and Helmann 2002). A *zosA* mutant exhibited significantly lower resistance to diamine, a thiol-specific oxidizing agent. A similar regulatory system involved in zinc uptake and resistance to  $H_2O_2$  was described in *L. lactis*. The two FNR-like (fumarate/nitrate reduction regulator) proteins, FlpA and FlpB, control the expression of a zinc-uptake system that increases cellular zinc, and they enhance the resistance to  $H_2O_2$  (Gostick et al. 1999).

On the other hand, excess zinc can inhibit protein function by blocking pivotal thiols or by competing with other metal ions for binding to the active sites of proteins. Zinc at high concentrations can also bind to negatively charged domains of proteins that are crucial for function. It was shown, for example, that zinc inhibits cytochrome *c* oxidase, presumably by binding to the negatively charged proton entry site of the enzyme (Aagaard and Brzezinski 2001). Clearly, zinc levels in the cell must be tightly regulated.

The first zinc-resistance protein was identified in the extremely metal-resistant bacterium *Ralstonia metallidurans*, followed by the CnrA protein from the same

bacterium (see Nies (2003) for review). They are members of the RND protein family, which was first described as a related group of bacterial transport proteins involved in heavy metal resistance (*R. metallidurans*), nodulation (*Mesorhizobium loti*), and cell division (*E. coli*) (Saier et al. 1994). This family has grown into a huge superfamily that includes seven protein families that can be found in all major kingdoms of life. In *R. metallidurans*, three genes are organized into the *czcCBA* operon. *CzcCBA* mediates resistance to  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$ , driven by the proton motive force. Similar systems are also involved in nickel and manganese efflux (Claverys 2001). These metal transporters belong to cluster nine of the family of ABC transporters, or ATP-binding cassette permeases. ABC transporters typically consist of a cytosolic metal-binding protein, a membrane permease, and an ATPase and can serve in the uptake as well as in the secretion of metal ions. ABC transporters can be found in the genomes of all bacterial species, but the function has been characterized only in a few cases.

Zinc homeostasis has so far received little attention in LAB, but transport systems for zinc uptake as well as for zinc efflux have been described for other Gram-positive bacteria (Hantke 2005). In *Streptococcus pneumoniae*, it has been proposed that the *adcCBA* operon encodes an ATP-binding cassette transporter for zinc uptake, and the *psa* one for manganese uptake (Dintilhac et al. 1997). A similar ABC-type manganese uptake system that is important for virulence has been described in *S. gordonii* (Dintilhac et al. 1997; Hantke 2005; Hazlett et al. 2003; Jakubovics et al. 2000; Janulczyk et al. 1999), and an ABC transporter of *Streptococcus pyogenes* has been shown to bind copper, iron, and zinc, but no transport studies were performed (Janulczyk et al. 1999). In *L. lactis* IL1403, *ZitSQP* is an ABC transporter putatively involved in high-affinity  $\text{Zn}^{2+}$  uptake (Bolotin et al. 2001). The ABC transporter-encoding genes, *zitSQP*, are organized into the putative *zitRSQP* operon, also encoding the *zitR* repressor. Sequence similarities of the putative *zitR* metalloregulator suggest that *zit* expression could be regulated by zinc present in the environment, as already shown for other zinc transport operons in Gram-positive bacteria (Dintilhac et al. 1997; Gaballa and Helmann 1998; Hantke 2005). Several zinc-uptake systems in bacteria have been shown to be under the control of a similar zinc-sensing Fur homolog, the zinc-uptake repressor Zur (Dalet et al. 1999; Gaballa and Helmann 2002; Lindsay and Foster 2001; Patzer and Hantke 2000).

An expression system  $P_{\text{Zn}}\text{zitR}$ , based on the regulatory signals ( $P_{\text{Zn}}$  promoter and *zitR* putative zinc repressor gene) of the *L. lactis* IL1403 *zit* operon, has been developed and shown to be highly inducible upon divalent cation starvation and strongly repressed in the presence of excess  $\text{Zn}^{2+}$ , thereby reinforcing the hypothesis of the involvement of the *zit* operon in  $\text{Zn}^{2+}$  high-affinity uptake and regulation in *L. lactis* IL1403 (Llull and Poquet 2004).

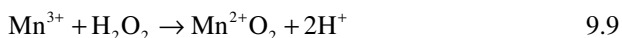
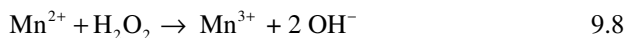
Zinc efflux in Gram-positive bacteria has received even less attention than zinc uptake. In *Streptococcus thermophilus* 4134, the chromosomal *cadC* and *cadA* genes were shown to constitute a cadmium/zinc resistance system (Schirawski et al. 2002). They are organized in an operon, and their transcription is cadmium-dependent in vivo. The predicted gene product of *cadA* is a CPx-type cadmium

efflux ATPase and that of *cadC* an ArsR-type regulatory protein. The two *cad* genes could also confer cadmium and zinc resistance to *L. lactis*. Clearly, the understanding of zinc homeostasis in LAB is still in its infancy, and considerable more work is required to understand the uptake, regulation, and secretion of zinc in these bacteria.

### 9.4.3 Response to Manganese

Cytoplasmic manganese can help to protect bacteria against oxidative stress, and the induction of manganese uptake by  $\text{H}_2\text{O}_2$  has been observed in many bacteria (Horsburgh et al. 2002b). *L. plantarum* can accumulate manganese to over 30 mM (Archibald and Duong 1984); uptake is accomplished by *mntA*, encoding an  $\text{Mn}^{2+}$ - and  $\text{Cd}^{2+}$ -transporting P-type ATPase (Hao et al. 1999). In *S. gordonii*, the *scaCBA* operon encodes an ABC-type manganese permease that is induced by low-ambient manganese via the ScaR repressor (Jakubovics et al. 2000). ABC-type manganese permeases as described in *S. gordonii* are also widespread in LAB (Claverys 2001). In addition to the ABC-type manganese permease MntABC, *Staphylococcus aureus* also possesses an Nramp-type manganese-uptake system, MntH (Horsburgh et al. 2002a). These systems are regulated by the manganese-dependent MntR repressor and the PerR oxidative stress regulator in a concerted fashion. Similar transporters and regulators have also been described in other bacteria (see Horsburgh et al. (2002b) for a review), but studies in LAB have remained scarce.

Manganese homeostasis plays a key role in many organisms, chiefly to defy oxidative stress and/or during infection of a human host (Jakubovics et al. 2000). In many Gram-positive bacteria, the major SOD that protects against oxidative stress is a manganese-containing enzyme (McEwan 2009). In addition to its role as a cofactor of SOD, manganese is able to directly protect against oxidative stress. In vitro, it has been shown that complexes of Mn(II) with bicarbonate can rapidly dismutate  $\text{H}_2\text{O}_2$  (Stadtman et al. 1990). The most recently proposed scheme for this reaction involves cycling between reactions (9.8) and (9.9):



The active species of manganese is an  $\text{Mn}^{2+}(\text{HCO}_3^-)_2$  complex in which  $\text{HCO}_3^-$  acts as an acceptor for protons (Tikhonov et al. 2006).  $\text{HCO}_3^-$  also lowers the redox potential of the Mn(II)–Mn(III) couple, which makes the reaction with  $\text{H}_2\text{O}_2$  more favorable. Nevertheless, the rate constants of these reactions are still far lower than those of enzymic manganese-dependent SODs and Mn catalases, and it was recently shown that imported Mn does not significantly scavenge  $\text{H}_2\text{O}_2$  in *E. coli* (Anjem

et al. 2009). Rather, the beneficial effects of manganese appear to lie in its ability to metallate mononuclear enzymes in lieu of iron. When the iron is not deeply buried in iron-loaded enzymes, it can engage in the Fenton reaction and cause oxidative stress. The substitution of such iron by manganese under oxidative stress conditions could thus prevent protein damage. In line with this concept, *E. coli* mutants that could not import manganese were found to suffer high rates of protein oxidation (Anjem et al. 2009). Clearly, the protection of bacteria against oxidative stress by manganese remains an interesting area of investigation for the future.

#### 9.4.4 Response to Nickel

Nickel is an essential trace nutrient for some bacteria, required at nanomolar concentrations. To date, nine nickel-containing enzymes are known: urease, NiFe-hydrogenase, carbon monoxide dehydrogenase, acetyl-CoA decarboxylase/synthase, methyl coenzyme M reductase, certain SODs, some glyoxylases, aci-reductone dioxigenase, and methylenediurease (Mulrooney and Hausinger 2003; Ragsdale 2009). None of these enzymes appears to play a role in LAB. Consequently, nickel is probably not an essential trace nutrient of these bacteria. Nickel in excess can induce oxidative stress in cells by cycling through the three redox states  $\text{Ni}^+$ ,  $\text{Ni}^{2+}$ , and  $\text{Ni}^{3+}$  (Costa et al. 2002). In organisms requiring nickel, it is taken up by dedicated nickel-uptake transporters, such as the NikABCDE import pump in *E. coli* (De Pina et al. 1999), or by high-affinity nickel/cobalt permeases (Eitinger et al. 2005). Members of these HoxN-type permeases have been identified in Gram-negative and Gram-positive bacteria (Eitinger and Mandrand-Berthelot 2000). Different Ni(II)-responsive metalloregulators that maintain nickel homeostasis in Gram-positive bacteria have been characterized. In *M. tuberculosis*, the transcription factor NmtR of the Ars/SmtB family inhibits the expression of the gene for NmtA, an ATP-dependent transporter responsible for the efflux of nickel and cobalt. NmtR tightly binds to the promoter region of the *nmtR* and *nmtA* genes and releases the DNA when nickel, or to a certain extent cobalt, is abundant (Cavet et al. 2002). A second nickel and cobalt sensor in *M. tuberculosis*, KmtA, represses the expression of a putative cation diffusion facilitator (CDF) metal exporter. NmtR and KmtA differ in their nickel-/cobalt-sensing affinity. It appears that first KmtR detects basal levels of cytosolic nickel or cobalt, which are then exported following the expression of the CDF transporter. Only when a higher threshold of these metals accumulates does NmtR sense them and allow expression of the P-type ATPase (Campbell et al. 2007). In *Streptomyces coelicolor*, the nickel-responsive regulator Nur, belonging to the Fur family, was characterized. This regulator represses the transcription of Fe-SOD and simultaneously induces the transcription of Ni-SOD under nickel stress (Ahn et al. 2006). It is not known how LAB respond to nickel stress. Natural environments are generally low in nickel and there may not have been a need for these organisms to evolve nickel detoxification systems. Indeed, the genome analysis of sequenced LAB does not reveal any genes that are obviously connected to nickel.

### 9.4.5 Response to Cobalt

Cobalt is a transition metal with the two naturally occurring oxidation states  $\text{Co}^{2+}$  and  $\text{Co}^{3+}$  and is primarily found in the corrin ring of coenzyme B12. To date, several noncorrin-cobalt-containing enzymes have been isolated and characterized (Kobayashi and Shimizu 1999). Cobalt undergoes redox chemistry and can thus participate in Fenton-type reactions, a fact making it potentially toxic at higher concentrations (Valko et al. 2005). Cobalt homeostasis is closely related to the homeostasis of nickel and other divalent ions. Both cobalt and nickel are taken up by the cell via secondary metal transporters with different ion preferences, ranging from strict selectivity for nickel through unbiased transport of both ions to a strong preference for cobalt (Eitinger et al. 2005; Komeda et al. 1997). In *S. aureus*, the zinc-/cobalt-responsive transcriptional repressor CzrA, which belongs to the ArsR/SmtB family, regulates the expression of the *czr* operon encoding a cobalt/zinc pump (Pennella et al. 2003). Transcriptional repressors with high-sequence identities to CzrA can be found in the genomes of many LAB, but no characterization of their function has been performed to date.

### 9.4.6 Response to Chromium

The widespread industrial use of the heavy metal chromium has caused it to be considered a serious environmental pollutant. It is mostly found in its trivalent or hexavalent forms in nature.  $\text{Cr}^{6+}$  is highly toxic to all forms of life, whereas  $\text{Cr}^{3+}$  is an essential micronutrient for many higher organisms (De Flora et al. 1990; Megharaj et al. 2003). However, for microorganisms and plants, chromium is nonessential. Chromate ( $\text{CrO}_4^{2-}$ ) crosses biological membranes by means of the sulfate-uptake pathway (Ramirez-Diaz et al. 2008). Inside the cell,  $\text{Cr}^{6+}$  is reduced to  $\text{Cr}^{3+}$ , a process in which free radicals may be formed (Liu and Shi 2001). Bacterial chromium-resistance systems related to plasmid genes usually encode membrane transporters that catalyze the efflux of chromate ions from the cytoplasm. The best-studied example is the *Pseudomonas aeruginosa* ChrA protein, which functions as a chemiosmotic pump that extrudes chromate from the cytoplasm using the proton motive force (Alvarez et al. 1999). A broad phylogenetic analysis for *chrA* transporter genes revealed homologous genes in bacteria, archaea, and fungi (Diaz-Perez et al. 2007). Several bacilli possess homologous gene sequences, but none of the known chromium-defense genes is present in the sequenced LAB genomes.

### 9.4.7 Response to Cadmium

Cadmium is a heavy metal with an oxidation state of +2. It is chemically similar to zinc and occurs naturally with zinc and lead in sulfide ores. Cadmium is not generally

believed to have a biological function; however, one enzyme (cadmium-carbonic anhydrase) incorporating cadmium under low-zinc conditions has been found in the marine diatom *Thalassiosira weissflogii* (Lane and Morel 2000). In spite of not being a Fenton metal, cadmium is capable of inducing oxidative stress in cell culture models and in experimental animals (Joseph et al. 2001; Nigam et al. 1999) and may exhibit its toxicity in microorganisms in a similar way. Cadmium is accumulated by cells via uptake systems responsible for essential cations. In Gram-positive bacteria, such as *B. subtilis*, *S. aureus*, or *L. plantarum*,  $\text{Cd}^{2+}$  competes for transport with  $\text{Mn}^{2+}$  (Archibald and Duong 1984; Burke and Pfister 1986; Tynecka et al. 1981). To prevent toxic effects by cadmium, active efflux mechanisms have evolved in prokaryotes. The best-characterized cadmium efflux system is that in the Gram-positive bacterium *S. aureus*, which consists of two plasmid-encoded genes, *cadA* and *cadC*. *CadA*, a CPx-type ATPase, catalyzes the efflux of  $\text{Cd}^{2+}$  (and probably also  $\text{Zn}^{2+}$ ) and *CadC* is a transcriptional repressor (Nucifora et al. 1989). *CadC* binds specifically to the *cad* operator DNA and is released by the addition of  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Bi}^{3+}$  (Endo and Silver 1995). Genetic analyses in *L. lactis* and *Oenococcus oeni* have shown the occurrence of similar plasmid-encoded cadmium-resistance systems in LAB (Bon et al. 2009; Liu et al. 1997). Additionally, cadmium may be pumped out of the cell by multidrug transporters. The ATP-binding cassette- (ABC) type multidrug transporters *LmrA* (*L. lactis*) and *OmrA* (*O. oeni*) could confer cadmium resistance to an *E. coli* mutant strain, which was hypersensitive to this heavy metal (Achard-Joris et al. 2005; Bourdineaud et al. 2004; Van Veen et al. 1996).

## 9.5 The Phosphate–Metal Connection

Most bacteria store phosphate in phosphate polymers of up to hundreds of residues called *polyphosphates*. It has been shown in a number of cases that polyphosphates are degraded under metal stress, such as by growth in the presence of lead or cadmium (Keasling 1997). Presumably, phosphate derived from the degradation of polyphosphate is exported as complexes with toxic metal ions, thereby detoxifying the cytoplasm. For example, an *E. coli* mutant defective in both polyphosphate kinase and polyphosphatase exhibited greatly increased cadmium sensitivity (Keasling and Hupf 1996). The extrusion of neutral metal phosphate complexes of the form  $\text{MeHPO}_4$  has, in fact, been directly demonstrated in *Acinetobacter johnsonii* and has been shown to generate electron-motif force (Van Veen et al. 1994b). Species of *Sulfolobus* have also been shown to accomplish high copper tolerance by the induction of polyphosphatase and secretion of copper phosphate (Remonsellez et al. 2006). The extrusion of metal–phosphate complexes takes place via the same Pit systems that also work in phosphate uptake (see ahead). Pit systems have been shown to catalyze the translocation of phosphate complexed to  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ , or  $\text{Mn}^{2+}$  (Van Veen et al. 1994a). Conceivably, complexes of phosphate with other divalent metal ions may also be translocated. LAB are generally not considered

to produce polyphosphate, but there are findings suggesting that at least some of them do (Benthin et al. 1994).

The phosphate transport systems that can participate in  $\text{MeHPO}_4$  extrusion can also serve in phosphate uptake under phosphate-limiting conditions. In *E. coli*, the low-affinity PitA and PitB phosphate transport systems were shown to catalyze the uptake of neutral metal–phosphate complexes (interestingly, PitA is nonfunctional in *E. coli* K-12 lab strains due to a mutation; (Harris et al. 2001)). The mutation of *E. coli pitA* conferred increased zinc resistance (Beard et al. 2000), and growth in the presence of zinc reduced the intracellular magnesium concentration and increased intracellular zinc, presumably due to competition between the two ions (Jackson et al. 2008). High-affinity phosphate uptake by *L. lactis* and related organisms is accomplished by an ATP-driven ABC-type transporter encoded by the *pstFEDCBA* operon. Mutations in *pstFEDCBA* were found to increase the resistance to copper and zinc by lowering the intracellular reactivity of these metals, which in turn also reduced the sensitivity of the cells to oxygen (Cesselin et al. 2009). This suggests that the *pst* system can (or must) also transport metal–phosphate complexes.

The observation of Pit- and Pst-catalyzed metal–phosphate cotransport is a surprising aspect of these transporters, which was not taken into consideration in most studies of either phosphate or metal transport. This masquerade may have disguised the true function of many transporters. The magnesium transporter CorA, which is ubiquitous in Gram-negative bacteria, may in fact be a metal–phosphate transporter, and the magnesium transporter MgtE, which also occurs in Gram-positive bacteria, may similarly be a metal–phosphate transporter. Clearly, much more work is required for a detailed understanding of bacterial metal transport.

**Acknowledgments** Some of the work described in this chapter has been supported by Grant 3100A0\_122551 from the Swiss National Foundation, a grant from the Swiss State Secretary for Education & Research, and a grant from the International Copper Association.

## References

- Aagaard A, Brzezinski P (2001) Zinc ions inhibit oxidation of cytochrome *c* oxidase by oxygen. *FEBS Lett* 494:157–160
- Achard-Joris M, van den Berg van Saparoea HB, Driessen AJ, Bourdineaud JP (2005) Heterologously expressed bacterial and human multidrug resistance proteins confer cadmium resistance to *Escherichia coli*. *Biochemistry* 44:5916–5922
- Ahn BE, Cha J, Lee EJ, Han AR, Thompson CJ, Roe JH (2006) Nur, a nickel-responsive regulator of the Fur family, regulates superoxide dismutases and nickel transport in *Streptomyces coelicolor*. *Mol Microbiol* 59:1848–1858
- Alvarez AH, Moreno-Sanchez R, Cervantes C (1999) Chromate efflux by means of the ChrA chromate resistance protein from *Pseudomonas aeruginosa*. *J Bacteriol* 181:7398–7400
- Andrews SC, Robinson AK, Rodriguez-Quinones F (2003) Bacterial iron homeostasis. *FEMS Microbiol Rev* 27:215–237
- Anjem A, Varghese S, Imlay JA (2009) Manganese import is a key element of the OxyR response to hydrogen peroxide in *Escherichia coli*. *Mol Microbiol* 72:844–858

- Archibald FS, Duong MN (1984) Manganese acquisition by *Lactobacillus plantarum*. *J Bacteriol* 158:1–8
- Arciero DM, Pierce BS, Hendrich MP, Hooper AB (2002) Nitrosocyanin, a red cupredoxin-like protein from *Nitrosomonas europaea*. *Biochemistry* 41:1703–1709
- Arguello JM, Gonzalez-Guerrero M (2008) Cu<sup>+</sup>-ATPases brake system. *Structure* 16:833–834
- Arnesano F, Banci L, Bertini I, Ciofi-Baffoni S, Molteni E, Huffman DL, O'Halloran TV (2002) Metallochaperones and metal-transporting ATPases: a comparative analysis of sequences and structures. *Genome Res* 12:255–271
- Axelsen KB, Palmgren MG (1998) Evolution of substrate specificities in the P-type ATPase superfamily. *J Mol Evol* 46:84–101
- Baichoo N, Wang T, Ye R, Helmann JD (2002) Global analysis of the *Bacillus subtilis* Fur regulon and the iron starvation stimulon. *Mol Microbiol* 45:1613–1629
- Balasubramanian R, Rosenzweig AC (2008) Copper methanobactin: a molecule whose time has come. *Curr Opin Chem Biol* 12:245–249
- Barkay T, Miller SM, Summers AO (2003) Bacterial mercury resistance from atoms to ecosystems. *FEMS Microbiol Rev* 27:355–384
- Barré O, Mourlane F, Solioz M (2007) Copper induction of lactate oxidase of *Lactococcus lactis*: a novel metal stress response. *J Bacteriol* 189:5947–5954
- Battistoni A (2003) Role of prokaryotic Cu, Zn superoxide dismutase in pathogenesis. *Biochem Soc Trans* 31:1326–1329
- Beard SJ, Hashim R, Wu G, Binet MR, Hughes MN, Poole RK (2000) Evidence for the transport of zinc(II) ions via the pit inorganic phosphate transport system in *Escherichia coli*. *FEMS Microbiol Lett* 184:231–235
- Benthin S, Nielsen J, Villadsen J (1994) Galactose expulsion during lactose metabolism in *Lactococcus lactis* subsp. *cremoris* FD1 due to dephosphorylation of intracellular galactose 6-phosphate. *Appl Environ Microbiol* 60:1254–1259
- Bird AJ (2008) Metallosensors, the ups and downs of gene regulation. *Adv Microb Physiol* 53:231–267
- Bolotin A, Wincker P, Mauger S, Jaillon O, Malarne K, Weissenbach J, Ehrlich SD, Sorokin A (2001) The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Res* 11:731–753
- Bon E, Delaherche A, Bilhere E, De Daruvar A, Lonvaud-Funel A, Le MC (2009) *Oenococcus oeni* genome plasticity is associated with fitness. *Appl Environ Microbiol* 75:2079–2090
- Bourdineaud JP, Nehme B, Tesse S, Lonvaud-Funel A (2004) A bacterial gene homologous to ABC transporters protect *Oenococcus oeni* from ethanol and other stress factors in wine. *Int J Food Microbiol* 92:1–14
- Brazeau BJ, Johnson BJ, Wilmot CM (2004) Copper-containing amine oxidases. *Biogenesis and catalysis; a structural perspective. Arch Biochem Biophys* 428:22–31
- Brooijmans RJW, Poolman B, Schuurman-Wolters GK, De Vos WM, Hugenholtz J (2007) Generation of a membrane potential by *Lactococcus lactis* through aerobic electron transport. *J Bacteriol* 189:5203–5209
- Bsat N, Helmann JD (1999) Interaction of *Bacillus subtilis* Fur (ferric uptake repressor) with the *dhb* operator *in vitro* and *in vivo*. *J Bacteriol* 181:4299–4307
- Burke BE, Pfister RM (1986) Cadmium transport by a Cd<sup>2+</sup>-sensitive and a Cd<sup>2+</sup>-resistant strain of *Bacillus subtilis*. *Can J Microbiol* 32:539–542
- Bury NR, Grosell M, Grover AK, Wood CM (1999) ATP-dependent silver transport across the basolateral membrane of rainbow trout gills. *Toxicol Appl Pharmacol* 159:1–8
- Campbell DR, Chapman KE, Waldron KJ, Tottey S, Kendall S, Cavallaro G, Andreini C, Hinds J, Stoker NG, Robinson NJ, Cavet JS (2007) Mycobacterial cells have dual nickel-cobalt sensors: sequence relationships and metal sites of metal-responsive repressors are not congruent. *J Biol Chem* 282:32298–32310
- Cantini F, Banci L, Solioz M (2009) The copper-responsive repressor CopR of *Lactococcus lactis* is a “winged helix” protein. *Biochem J* 417:493–499
- Cavet JS, Meng W, Pennella MA, Appelhoff RJ, Giedroc DP, Robinson NJ (2002) A nickel-cobalt-sensing ArsR-SmtB family repressor. Contributions of cytosol and effector binding sites to metal selectivity. *J Biol Chem* 277:38441–38448

- Cavet JS, Borrelly GP, Robinson NJ (2003) Zn, Cu and Co in cyanobacteria: selective control of metal availability. *FEMS Microbiol Rev* 27:165–181
- Cesselin B, Ali D, Gratadoux JJ, Gaudu P, Duwat P, Gruss A, El KM (2009) Inactivation of the *Lactococcus lactis* high affinity phosphate transporter confers oxygen and thiol resistance and alters metal homeostasis. *Microbiology* 155:2274–2281
- Cha JS, Cooksey DA (1991) Copper resistance in *Pseudomonas syringae* mediated by periplasmic and outer membrane proteins. *Proc Natl Acad Sci USA* 88:8915–8919
- Chan SI, Chen KH, Yu SS, Chen CL, Kuo SS (2004) Toward delineating the structure and function of the particulate methane monooxygenase from methanotrophic bacteria. *Biochemistry* 43:4421–4430
- 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* 301:1383–1387
- Chillappagari S, Miethke M, Trip H, Kuipers OP, Marahiel MA (2009) Copper acquisition is mediated by YcnJ and regulated by YcnK and CsoR in *Bacillus subtilis*. *J Bacteriol* 191:2362–2370
- Claverys JP (2001) A new family of high-affinity ABC manganese and zinc permeases. *Res Microbiol* 152:231–243
- Cobine P, Wickramasinghe WA, Harrison MD, Weber T, Solioz M, Dameron CT (1999) The *Enterococcus hirae* copper chaperone CopZ delivers copper(I) to the CopY repressor. *FEBS Lett* 445:27–30
- Cobine P, Jones CE, Wickramasinghe WA, Solioz M, Dameron CT (2002a) Interaction of copper binding proteins from *Enterococcus hirae*. In: Massaro EJ (Ed.), *Handbook of copper pharmacology and toxicology*. Humana Press, Totowa, pp. 177–186
- Cobine PA, George GN, Jones CE, Wickramasinghe WA, Solioz M, Dameron CT (2002b) Copper transfer from the Cu(I) chaperone, CopZ, to the repressor, Zn(II)CopY: Metal coordination environments and protein interactions. *Biochemistry* 41:5822–5829
- Cobine PA, Jones CE, Dameron CT (2002c) Role for zinc(II) in the copper(I) regulated protein CopY. *J Inorg Biochem* 88:192–196
- Coleman JE (1998) Zinc enzymes. *Curr Opin Chem Biol* 2:222–234
- Costa M, Salnikow K, Sutherland JE, Broday L, Peng W, Zhang Q, Kluz T (2002) The role of oxidative stress in nickel and chromate genotoxicity. *Mol Cell Biochem* 234–235:265–275
- Crichton RR, Pierre J-L (2001) Old iron, young copper: from Mars to Venus. *Biometals* 14:99–112
- Dalet K, Gouin E, Cenatiempo Y, Cossart P, Hechard Y (1999) Characterisation of a new operon encoding a Zur-like protein and an associated ABC zinc permease in *Listeria monocytogenes*. *FEMS Microbiol Lett* 174:111–116
- Davis AV, O'Halloran TV (2008) A place for thioether chemistry in cellular copper ion recognition and trafficking. *Nat Chem Biol* 4:148–151
- De Flora S, Bagnasco M, Serra D, Zancchi P (1990) Genotoxicity of chromium compounds. A review. *Mutat Res* 238:99–172
- De Pina K, Desjardin V, Mandrand-Berthelot MA, Giordano G, Wu LF (1999) Isolation and characterization of the *nikR* gene encoding a nickel-responsive regulator in *Escherichia coli*. *J Bacteriol* 181:670–674
- 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: 6215–6227
- Dintilhac A, Alloing G, Granadel C, Claverys JP (1997) Competence and virulence of *Streptococcus pneumoniae*: Adc and PsaA mutants exhibit a requirement for Zn and Mn resulting from inactivation of putative ABC metal permeases. *Mol Microbiol* 25:727–739
- Dunn KL, Farrant JL, Langford PR, Kroll JS (2003) Bacterial [Cu,Zn]-cofactored superoxide dismutase protects opsonized, encapsulated *Neisseria meningitidis* from phagocytosis by human monocytes/macrophages. *Infect Immun* 71:1604–1607
- Eitinger T, Mandrand-Berthelot MA (2000) Nickel transport systems in microorganisms. *Arch Microbiol* 173:1–9

- Eitinger T, Suhr J, Moore L, Smith JA (2005) Secondary transporters for nickel and cobalt ions: theme and variations. *Biometals* 18:399–405
- Ellis MJ, Grossmann JG, Eady RR, Hasnain SS (2007) Genomic analysis reveals widespread occurrence of new classes of copper nitrite reductases. *J Biol Inorg Chem* 12:1119–1127
- Endo G, Silver S (1995) CadC, the transcriptional regulatory protein of the cadmium resistance system of *Staphylococcus aureus* plasmid pI258. *J Bacteriol* 177:4437–4441
- Gaballa A, Helmann JD (1998) Identification of a zinc-specific metalloregulatory protein, Zur, controlling zinc transport operons in *Bacillus subtilis*. *J Bacteriol* 180:5815–5821
- Gaballa A, Helmann JD (2002) A peroxide-induced zinc uptake system plays an important role in protection against oxidative stress in *Bacillus subtilis*. *Mol Microbiol* 45:997–1005
- Galvez A, Abriouel H, Lopez RL, Ben ON (2007) Bacteriocin-based strategies for food biopreservation. *Int J Food Microbiol* 120:51–70
- Garcia-Castellanos R, Mallorqui-Fernandez G, Marrero A, Potempa J, Coll M, Gomis-Ruth FX (2004) On the transcriptional regulation of methicillin resistance: MecI repressor in complex with its operator. *J Biol Chem* 279:17888–17896
- Gonzalez-Guerrero M, Arguello JM (2008) Mechanism of Cu<sup>+</sup>-transporting ATPases: soluble Cu<sup>+</sup> chaperones directly transfer Cu<sup>+</sup> to transmembrane transport sites. *Proc Natl Acad Sci USA* 105:5992–5997
- Gostick DO, Griffin HG, Shearman CA, Scott C, Green J, Gasson MJ, Guest JR (1999) Two operons that encode FNR-like proteins in *Lactococcus lactis*. *Mol Microbiol* 31:1523–1535
- Gupta A, Matsui K, Lo JF, Silver S (1999) Molecular basis for resistance to silver cations in *Salmonella*. *Nat Med* 5:183–188
- Hanson SR, Donley SA, Linder MC (2001) Transport of silver in virgin and lactating rats and relation to copper. *J Trace Elem Med Biol* 15:243–253
- Hantke K (2005) Bacterial zinc uptake and regulators. *Curr Opin Microbiol* 8:196–202
- Hao Z, Reiske HR, Wilson DB (1999) Characterization of cadmium uptake in *Lactobacillus plantarum* and isolation of cadmium and manganese uptake mutants. *Appl Environ Microbiol* 65:4741–4745
- Harris RM, Webb DC, Howitt SM, Cox GB (2001) Characterization of PitA and PitB from *Escherichia coli*. *J Bacteriol* 183:5008–5014
- Hasman H, Kempf I, Chidaine B, Cariolet R, Ersboll AK, Houe H, Bruun Hansen HC, Aarestrup FM (2006) Copper resistance in *Enterococcus faecium*, mediated by the *tcpB* gene, is selected by supplementation of pig feed with copper sulfate. *Appl Environ Microbiol* 72:5784–5789
- Hazlett KR, Rusnak F, Kehres DG, Bearden SW, La Vake CJ, La Vake ME, Maguire ME, Perry RD, Radolf JD (2003) The *Treponema pallidum* *tro* operon encodes a multiple metal transporter, a zinc-dependent transcriptional repressor, and a semi-autonomously expressed phosphoglycerate mutase. *J Biol Chem* 278:20687–20694
- Himeno T, Imanaka T, Aiba S (1986) Nucleotide sequence of the penicillinase repressor gene *penI* of *Bacillus licheniformis* and regulation of *penP* and *penI* by the repressor. *J Bacteriol* 168:1128–1132
- Horsburgh MJ, Wharton SJ, Cox AG, Ingham E, Peacock S, Foster SJ (2002a) MntR modulates expression of the PerR regulon and superoxide resistance in *Staphylococcus aureus* through control of manganese uptake. *Mol Microbiol* 44:1269–1286
- Horsburgh MJ, Wharton SJ, Karavolos M, Foster SJ (2002b) Manganese: elemental defence for a life with oxygen. *Trends Microbiol* 10:496–501
- Huffman DL, O'Halloran TV (2001) Function, structure, and mechanism of intracellular copper trafficking proteins. *Annu Rev Biochem* 70:677–701
- Hullo MF, Moszer I, Danchin A, Martin-Verstraete I (2001) CotA of *Bacillus subtilis* is a copper-dependent laccase. *J Bacteriol* 183:5426–5430
- Imbert M, Blondeau R (1998) On the iron requirement of lactobacilli grown in chemically defined medium. *Curr Microbiol* 37:64–66
- Jackson RJ, Binet MR, Lee LJ, Ma R, Graham AI, McLeod CW, Poole RK (2008) Expression of the PitA phosphate/metal transporter of *Escherichia coli* is responsive to zinc and inorganic phosphate levels. *FEMS Microbiol Lett* 289:219–224
- Jakubovics NS, Smith AW, Jenkinson HF (2000) Expression of the virulence-related Sca (Mn<sup>2+</sup>) permease in *Streptococcus gordonii* is regulated by a diphtheria toxin metalloregulator-like protein ScaR. *Mol Microbiol* 38:140–153

- Janulczyk R, Pallon J, Bjorck L (1999) Identification and characterization of a *Streptococcus pyogenes* ABC transporter with multiple specificity for metal cations. *Mol Microbiol* 34:596–606
- Joseph P, Muchnok TK, Klshis ML, Roberts JR, Antonini JM, Whong WZ, Ong T (2001) Cadmium-induced cell transformation and tumorigenesis are associated with transcriptional activation of c-fos, c-jun, and c-myc proto-oncogenes: role of cellular calcium and reactive oxygen species. *Toxicol Sci* 61:295–303
- Kaim W, Rall J (1996) Copper – a “modern” bioelement. *Angew Chem Int Ed Engl* 35:43–60
- Kanamaru K, Kashiwagi S, Mizuno T (1994) A copper-transporting P-type ATPase found in the thylakoid membrane of the cyanobacterium *Synechococcus* species PCC7942. *Mol Microbiol* 13:369–377
- Kaneko T, Takahashi M, Suzuki H (1990) Acetoin fermentation by citrate-positive *Lactococcus lactis* subsp. *lactis* 3022 grown aerobically in the presence of hemin or Cu. *Appl Environ Microbiol* 56:2644–2649
- Karlin KD (1993) Metalloenzymes, structural motifs, and inorganic models. *Science* 261:701–708
- Kasting JF, Siefert JL (2002) Life and the evolution of Earth’s atmosphere. *Science* 296:1066–1068
- Keasling JD (1997) Regulation of intracellular toxic metals and other cations by hydrolysis of polyphosphate. *Ann NY Acad Sci* 829:242–249
- Keasling JD, Hupf GA (1996) Genetic manipulation of polyphosphate metabolism affects cadmium tolerance in *Escherichia coli*. *Appl Microbiol Biotechnol* 62:743–746
- Kehres DG, Zaharik ML, Finlay BB, Maguire ME (2000) The NRAMP proteins of *Salmonella typhimurium* and *Escherichia coli* are selective manganese transporters involved in the response to reactive oxygen. *Mol Microbiol* 36:1085–1100
- Kiermeier F, Kyrein HJ (1971) Einfluß des Kupfers auf den Acetoin-Diacetyl- und Pyruvatgehalt von Käse [in German]. *Z Lebensmittelunters -Forschung A* 147:128–133
- Kihlken MA, Leech AP, Le Brun NE (2002) Copper-mediated dimerization of CopZ, a predicted copper chaperone from *Bacillus subtilis*. *Biochem J* 368:729–739
- Kihlken MA, Singleton C, Le Brun NE (2008) Distinct characteristics of Ag<sup>+</sup> and Cd<sup>2+</sup> binding to CopZ from *Bacillus subtilis*. *J Biol Inorg Chem* 13:1011–1023
- Kim BE, Nevitt T, Thiele DJ (2008) Mechanisms for copper acquisition, distribution and regulation. *Nat Chem Biol* 4:176–185
- Kim HJ, Graham DW, DiSpirito AA, Alterman MA, Galeva N, Larive CK, Asunskis D, Sherwood PM (2004) Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science* 305:1612–1615
- Kobayashi M, Shimizu S (1999) Cobalt proteins. *Eur J Biochem* 261:1–9
- Komeda H, Kobayashi M, Shimizu S (1997) A novel transporter involved in cobalt uptake. *Proc Natl Acad Sci USA* 94:36–41
- Kuper J, Llamas A, Hecht HJ, Mendel RR, Schwarz G (2004) Structure of the molybdopterin-bound Cnx1G domain links molybdenum and copper metabolism. *Nature* 430:803–806
- Lane TW, Morel FM (2000) A biological function for cadmium in marine diatoms. *Proc Natl Acad Sci USA* 97:4627–4631
- Lindsay JA, Foster SJ (2001) *zur*: a Zn<sup>2+</sup>-responsive regulatory element of *Staphylococcus aureus*. *Microbiology* 147:1259–1266
- Liu CQ, Khunajakr N, Chia LG, Deng YM, Charoenchai P, Dunn NW (1997) Genetic analysis of regions involved in replication and cadmium resistance of the plasmid pND302 from *Lactococcus lactis*. *Plasmid* 38:79–90
- Liu KJ, Shi X (2001) *In vivo* reduction of chromium (VI) and its related free radical generation. *Mol Cell Biochem* 222:41–47
- Liu T, Ramesh A, Ma Z, Ward SK, Zhang L, George GN, Talaat AM, Sacchettini JC, Giedroc DP (2007) CsoR is a novel *Mycobacterium tuberculosis* copper-sensing transcriptional regulator. *Nat Chem Biol* 3:60–68
- Llull D, Poquet I (2004) New expression system tightly controlled by zinc availability in *Lactococcus lactis*. *Appl Environ Microbiol* 70:5398–5406

- Lopez-Serrano D, Solano F, Sanchez-Amat A (2004) Identification of an operon involved in tyrosinase activity and melanin synthesis in *Marinomonas mediterranea*. *Gene* 342:179–187
- Lu ZH, Solioz M (2001) Copper-induced proteolysis of the CopZ copper chaperone of *Enterococcus hirae*. *J Biol Chem* 276:47822–47827
- Lutsenko S, Kaplan JH (1995) Organization of P-type ATPases: significance of structural diversity. *Biochemistry* 34:15607–15613
- Ma Z, Cowart D, Scott R, Giedroc DP (2009) Molecular insights into the metal selectivity of the Cu(I)-sensing repressor CsoR from *Bacillus subtilis*. *Biochemistry* 48:3325–3334
- Macomber L, Imlay JA (2009) The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. *Proc Natl Acad Sci USA* 106:8344–8349
- Macomber L, Rensing C, Imlay JA (2007) Intracellular copper does not catalyze the formation of oxidative DNA damage in *Escherichia coli*. *J Bacteriol* 189:1616–1626
- Magnani D, Barré O, Gerber SD, Solioz M (2008) Characterization of the CopR regulon of *Lactococcus lactis* IL1403. *J Bacteriol* 190:536–545
- Makui H, Roig E, Cole ST, Helmann JD, Gros P, Cellier MF (2000) Identification of the *Escherichia coli* K-12 Nramp orthologue (MntH) as a selective divalent metal ion transporter. *Mol Microbiol* 35:1065–1078
- Marty-Teyssset C, de la Torre F, Garel J (2000) Increased production of hydrogen peroxide by *Lactobacillus delbrueckii* subsp. *bulgaricus* upon aeration: involvement of an NADH oxidase in oxidative stress. *Appl Environ Microbiol* 66:262–267
- McEwan AG (2009) New insights into the protective effect of manganese against oxidative stress. *Mol Microbiol* 72:812–814
- Megharaj M, Avudainayagam S, Naidu R (2003) Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. *Curr Microbiol* 47:51–54
- Mitrakul K, Loo CY, Hughes CV, Ganeshkumar N (2004) Role of a *Streptococcus gordonii* copper-transport operon, *copYAZ*, in biofilm detachment. *Oral Microbiol Immunol* 19:395–402
- Miyoshi A, Rochat T, Gratadoux JJ, Le Loir Y, Oliveira SC, Langella P, Azevedo V (2003) Oxidative stress in *Lactococcus lactis*. *Genet Mol Res* 2:348–359
- Moore CM, Helmann JD (2005) Metal ion homeostasis in *Bacillus subtilis*. *Curr Opin Microbiol* 8:188–195
- Mulrooney SB, Hausinger RP (2003) Nickel uptake and utilization by microorganisms. *FEMS Microbiol Rev* 27:239–261
- Multhaup G, Strausak D, Bissig K-D, Solioz M (2001) Interaction of the CopZ copper chaperone with the CopA copper ATPase of *Enterococcus hirae* assessed by surface plasmon resonance. *Biochem Biophys Res Commun* 288:172–177
- Narindrasorasak S, Zhang X, Roberts EA, Sarkar B (2004) Comparative analysis of metal binding characteristics of copper chaperone proteins, Atx1 and ATOX1. *Bioinorg Chem Appl* 2:105–123
- Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. *J Biol Chem* 270:26723–26726
- Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiol Rev* 27:313–339
- Nigam D, Shukla GS, Agarwal AK (1999) Glutathione depletion and oxidative damage in mitochondria following exposure to cadmium in rat liver and kidney. *Toxicol Lett* 106:151–157
- Nucifora G, Chu L, Misra TK, Silver S (1989) Cadmium resistance from *Staphylococcus aureus* plasmid pI258 *cadA* gene results from a cadmium-efflux ATPase. *Proc Natl Acad Sci USA* 86:3544–3548
- Odermatt A, Solioz M (1995) Two *trans*-acting metalloregulatory proteins controlling expression of the copper-ATPases of *Enterococcus hirae*. *J Biol Chem* 270:4349–4354
- Odermatt A, Suter H, Krapp R, Solioz M (1992) An ATPase operon involved in copper resistance by *Enterococcus hirae*. *Ann NY Acad Sci* 671:484–486
- Odermatt A, Krapp R, Solioz M (1994) Induction of the putative copper ATPases, CopA and CopB, of *Enterococcus hirae* by Ag<sup>+</sup> and Cu<sup>2+</sup>, and Ag<sup>+</sup> extrusion by CopB. *Biochem Biophys Res Commun* 202:44–48

- Outten CE, O'Halloran TV (2001) Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science* 292:2488–2492
- Outten FW, Outten CE, Hale J, O'Halloran TV (2000) Transcriptional activation of an *Escherichia coli* copper efflux regulon by the chromosomal MerR homologue, CueR. *J Biol Chem* 275:31024–31029
- Outten FW, Huffman DL, Hale JA, O'Halloran TV (2001) The independent *cue* and *cus* systems confer copper tolerance during aerobic and anaerobic growth in *Escherichia coli*. *J Biol Chem* 276:30670–30677
- Patzter SI, Hantke K (2000) The zinc-responsive regulator Zur and its control of the *znu* gene cluster encoding the ZnuABC zinc uptake system in *Escherichia coli*. *J Biol Chem* 275:24321–24332
- Pedersen PL, Carafoli E (1987) Ion motive ATPases. I. Ubiquity, properties, and significance to cell function. *Trends Biochem Sci* 12:146–150
- Penaud S, Fernandez A, Boudebouze S, Ehrlich SD, Maguin E, van de Guchte M (2006) Induction of heavy-metal-transporting CPX-type ATPases during acid adaptation in *Lactobacillus bulgaricus*. *Appl Environ Microbiol* 72:7445–7454
- Pennella MA, Shokes JE, Cosper NJ, Scott RA, Giedroc DP (2003) Structural elements of metal selectivity in metal sensor proteins. *Proc Natl Acad Sci USA* 100:3713–3718
- Perez JM, Calderon IL, Arenas FA, Fuentes DE, Pradenas GA, Fuentes EL, Sandoval JM, Castro ME, Elias AO, Vasquez CC (2007) Bacterial toxicity of potassium tellurite: unveiling an ancient enigma. *PLoS ONE* 2:e211
- Portmann R, Magnani D, Stoyanov JV, Schmechel A, Multhaup G, Solioz M (2004) Interaction kinetics of the copper-responsive CopY repressor with the *cop* promoter of *Enterococcus hirae*. *J Biol Inorg Chem* 9:396–402
- Portmann R, Poulsen KR, Wimmer R, Solioz M (2006) CopY-like copper inducible repressors are putative “winged helix” proteins. *Biometals* 19:61–70
- Powell SR (2000) The antioxidant properties of zinc. *J Nutr* 130:1447S–1454S
- Pufahl RA, Singer CP, Peariso KL, Lin S, Schmidt PJ, Fahrni CJ, Culotta VC, Penner-Hahn JE, O'Halloran TV (1997) Metal ion chaperone function of the soluble Cu(I) receptor Atx1. *Science* 278:853–856
- Radford DS, Kihlken MA, Borrelly GP, Harwood CR, Le Brun NE, Cavet JS (2003) CopZ from *Bacillus subtilis* interacts *in vivo* with a copper exporting CPX-type ATPase CopA. *FEMS Microbiol Lett* 220:105–112
- Ragsdale SW (2009) Nickel-based enzyme systems. *J Biol Chem* 284:18571–18575
- Ramirez-Diaz MI, Diaz-Perez C, Vargas E, Riveros-Rosas H, Campos-Garcia J, Cervantes C (2008) Mechanisms of bacterial resistance to chromium compounds. *Biometals* 21:321–332
- Rapisarda VA, Chehin RN, De Las Rivas J, Rodriguez-Montelongo L, Farias RN, Massa EM (2002) Evidence for Cu(I)-thiolate ligation and prediction of a putative copper-binding site in the *Escherichia coli* OADH dehydrogenase-2. *Arch Biochem Biophys* 405:87–94
- Reeve WG, Tiwari RP, Kale NB, Dilworth MJ, Glenn AR (2002) ActP controls copper homeostasis in *Rhizobium leguminosarum* bv. *viciae* and *Sinorhizobium meliloti* preventing low pH-induced copper toxicity. *Mol Microbiol* 43:981–991
- Remonsellez F, Orell A, Jerez CA (2006) Copper tolerance of the thermoacidophilic archaeon *Sulfolobus metallicus*: possible role of polyphosphate metabolism. *Microbiology* 152:59–66
- Richer E, Courville P, Bergevin I, Cellier MF (2003) Horizontal gene transfer of “prototype” Nramp in bacteria. *J Mol Evol* 57:363–376
- Ridge PG, Zhang Y, Gladyshev VN (2008) Comparative genomic analyses of copper transporters and cuproproteomes reveal evolutionary dynamics of copper utilization and its link to oxygen. *PLoS One* 3:e1378
- Rochat T, Gratadoux JJ, Gruss A, Corthier G, Maguin E, Langella P, van de Guchte M (2006) Production of a heterologous nonheme catalase by *Lactobacillus casei*: an efficient tool for removal of H<sub>2</sub>O<sub>2</sub> and protection of *Lactobacillus bulgaricus* from oxidative stress in milk. *Appl Environ Microbiol* 72:5143–5149
- Rodriguez-Montelongo L, Volentini SI, Farias RN, Massa EM, Rapisarda VA (2006) The Cu(II)-reductase NADH dehydrogenase-2 of *Escherichia coli* improves the bacterial growth in

- extreme copper concentrations and increases the resistance to the damage caused by copper and hydroperoxide. *Arch Biochem Biophys* 451:1–7
- Rosenzweig AC (2001) Copper delivery by metallochaperone proteins. *Acc Chem Res* 34:119–128
- Saier MH Jr, Tam R, Reizer A, Reizer J (1994) Two novel families of bacterial membrane proteins concerned with nodulation, cell division and transport. *Mol Microbiol* 11:841–847
- Schirawski J, Hagens W, Fitzgerald GF, Van Sinderen D (2002) Molecular characterization of cadmium resistance in *Streptococcus thermophilus* strain 4134: an example of lateral gene transfer. *Appl Environ Microbiol* 68:5508–5516
- Schwarz G, Mendel RR (2006) Molybdenum cofactor biosynthesis and molybdenum enzymes. *Annu Rev Plant Biol* 57:623–647
- Scott C, Rawsthorne H, Upadhyay M, Shearman CA, Gasson MJ, Guest JR, Green J (2000) Zinc uptake, oxidative stress and the FNR-like proteins of *Lactococcus lactis*. *FEMS Microbiol Lett* 192:85–89
- Sharma VK, Hackbarth CJ, Dickinson TM, Archer GL (1998) Interaction of native and mutant MecI repressors with sequences that regulate *mecA*, the gene encoding penicillin binding protein 2a in methicillin-resistant staphylococci. *J Bacteriol* 180:2160–2166
- Singleton C, Le Brun NE (2009) The N-terminal soluble domains of *Bacillus subtilis* CopA exhibit a high affinity and capacity for Cu(I) ions. *Dalton Trans* 28:688–696
- Singleton C, Banci L, Ciofi-Baffoni S, Tenori L, Kihlken MA, Boetzel R, Le Brun NE (2008) Structure and Cu(I)-binding properties of the N-terminal soluble domains of *Bacillus subtilis* CopA. *Biochem J* 411:571–579
- Solioz M, Odermatt A (1995) Copper and silver transport by CopB-ATPase in membrane vesicles of *Enterococcus hirae*. *J Biol Chem* 270:9217–9221
- Solioz M, Odermatt A, Krapp R (1994) Copper pumping ATPases: common concepts in bacteria and man. *FEBS Lett* 346:44–47
- Solioz M, Stoyanov JV (2003) Copper homeostasis in *Enterococcus hirae*. *FEMS Microbiol Rev* 27:183–195
- Solioz M, Vulpe C (1996) CPx-type ATPases: a class of P-type ATPases that pump heavy metals. *Trends Biochem Sci* 21:237–241
- Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci* 275:177–182
- Stadtman ER, Berlett BS, Chock PB (1990) Manganese-dependent disproportionation of hydrogen peroxide in bicarbonate buffer. *Proc Natl Acad Sci USA* 87:384–388
- Steffen C, Eberhad P, Bosset JM, Rüegg M (2009) Swiss-type varieties. In: Fox PF (Ed.) *Cheese: chemistry, physics and microbiology*. Aspen Publishers, Gaithersburg, pp. 83–110
- Stoyanov JV, Magnani D, Solioz M (2003) Measurement of cytoplasmic copper, silver, and gold with a *lux* biosensor shows copper and silver, but not gold, efflux by the CopA ATPase of *Escherichia coli*. *FEBS Lett* 546:391–394
- Strausak D, Solioz M (1997) CopY is a copper-inducible repressor of the *Enterococcus hirae* copper ATPases. *J Biol Chem* 272:8932–8936
- Sun HW, Plapp BV (1992) Progressive sequence alignment and molecular evolution of the Zn-containing alcohol dehydrogenase family. *J Mol Evol* 34:522–535
- Tikhonov KG, Zastrizhnaya OM, Kozlov YN, Klimov VV (2006) Composition and catalase-like activity of Mn(II)-bicarbonate complexes. *Biochemistry (Mosc)* 71:1270–1277
- Totley S, Rich PR, Rondet SA, Robinson NJ (2001) Two Menkes-type atpases supply copper for photosynthesis in *Synechocystis* PCC 6803. *J Biol Chem* 276:19999–20004
- Totley S, Harvie DR, Robinson NJ (2005) Understanding how cells allocate metals using metal sensors and metallochaperones. *Acc Chem Res* 38:775–783
- Toyoshima C, Mizutani T (2004) Crystal structure of the calcium pump with a bound ATP analogue. *Nature* 430:529–535
- Toyoshima C, Nomura H, Sugita Y (2003) Crystal structures of Ca<sup>2+</sup>-ATPase in various physiological states. *Ann NY Acad Sci* 986:1–8
- Tsai TY, Lee YH (1998) Roles of copper ligands in the activation and secretion of *Streptomyces* tyrosinase. *J Biol Chem* 273:19243–19250

- Turner MS, Tan YP, Giffard PM (2007) Inactivation of an iron transporter in *Lactococcus lactis* results in resistance to tellurite and oxidative stress. *Appl Environ Microbiol* 73:6144–6149
- Tynecka Z, Gos Z, Zajac J (1981) Reduced cadmium transport determined by a resistance plasmid in *Staphylococcus aureus*. *J Bacteriol* 147:305–312
- Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. *Curr Med Chem* 12:1161–1208
- Vallee BL, Falchuk KH (1993) The biochemical basis of zinc physiology. *Physiol Rev* 73:79–118
- Van Melckebeke H, Vreuls C, Gans P, Filee P, Llabres G, Joris B, Simorre JP (2003) Solution structural study of BlaI: implications for the repression of genes involved in  $\beta$ -lactam antibiotic resistance. *J Mol Biol* 333:711–720
- Van Veen HW, Abee T, Kortstee GJ, Konings WN, Zehnder AJ (1994a) Translocation of metal phosphate via the phosphate inorganic transport system of *Escherichia coli*. *Biochemistry* 33:1766–1770
- Van Veen HW, Abee T, Kortstee GJ, Pereira H, Konings WN, Zehnder AJ (1994b) Generation of a proton motive force by the excretion of metal-phosphate in the polyphosphate-accumulating *Acinetobacter johnsonii* strain 210A. *J Biol Chem* 269:29509–29514
- Van Veen HW, Venema K, Bolhuis H, Oussenko I, Kok J, Poolman B, Driessen AJ, Konings WN (1996) Multidrug resistance mediated by a bacterial homolog of the human multidrug transporter MDR1. *Proc Natl Acad Sci USA* 93:10668–10672
- Vats N, Lee SF (2001) Characterization of a copper-transport operon, *copYAZ*, from *Streptococcus mutans*. *Microbiology* 147:653–662
- Weinberg ED (1997) The Lactobacillus anomaly: total iron abstinence. *Perspect Biol Med* 40:578–583
- Wimmer R, Herrmann T, Solioz M, Wüthrich K (1999) NMR structure and metal interactions of the CopZ copper chaperone. *J Biol Chem* 274:22597–22603
- Wittman V, Wong HC (1988) Regulation of the penicillinase genes of *Bacillus licheniformis*: interaction of the *pen* repressor with its operators. *J Bacteriol* 170:3206–3212
- Wunderli-Ye H, Solioz M (2001) Purification and functional analysis of the copper ATPase CopA of *Enterococcus hirae*. *Biochem Biophys Res Commun* 280:713–719
- Zhang Y, Gladyshev VN (2008) Molybdoproteomes and evolution of molybdenum utilization. *J Mol Biol* 379:881–899
- Zhang Y, Turanov AA, Hatfield DL, Gladyshev VN (2008) *In silico* identification of genes involved in selenium metabolism: evidence for a third selenium utilization trait. *BMC Genomics* 9:251
- Zhang Y, Rodionov DA, Gelfand MS, Gladyshev VN (2009) Comparative genomic analyses of nickel, cobalt and vitamin B12 utilization. *BMC Genomics* 10:78–103