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Review

Copper resistance mechanisms in plant pathogenic bacteria

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Summary. Copper is an essential element for microbes as it is involved in many redox reactions. Numerous resistance systems have been evolved in microbes to maintain copper homeostasis under copper stress conditions. These systems are responsible for the influx and efflux of copper ions in the cells. In phytopathogenic bacteria, copper ions play essential roles during disease development in plants. Copper-based chemicals are extensively used for control of diseases caused by bacteria, which leads to induced pathogen resistance derived from various copper resistance systems. Previous studies have shown that copper ions are harnessed by host plants to protect against bacterial infections, triggering immune responses through activation of defence signalling pathways. Thus, it was anticipated that bacterial copper resistance could play an alternative role in adaptation to plant immunity. This review summarizes current knowledge of copper resistance systems in plant pathogenic bacteria, which may provide a new perspective of molecular mechanisms associated with bacterial adaptation in host plants.

Keywords. Resistance systems, stress conditions, signalling pathways, bacterial copper resistance, plant immunity.

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INTRODUCTION

In nature, plants are always under threats from pests and pathogens. Pathogenic bacteria are a major cause of diseases in diverse plants, resulting in negative effects on plant growth and crop yields.

Utilization of copper is essential in living organisms. Due to easy conversion process between reduced Cu(I) and oxidized Cu(II) with low energy consumption, copper serves as a cofactor for many key enzymes that are involved in essential biochemical and physiological processes, including electron transport, oxidative stress response, denitrification, respiration and photosynthesis (Arredondo and Nunez, 2005; Turski and Thiele, 2009; Festa and Thiele, 2011; Argüello *et al*., 2013; Rensing and McDevitt, 2013;). However, copper ions are toxic when exceeding a threshold value within cells (Adrees *et al*., 2015; Husak *et al*., 2018; Kalita *et al*., 2018). The toxicity mechanisms have been attributed to generation of highly reactive hydroxyl radicals via Fenton and Haber-Weiss reactions (Liochev and Fridovich, 2002), affecting

biomolecules such as peptides, DNA, and lipids (Freinbichler *et al*., 2011). Excess copper can also bind to adventitious sites in proteins, disrupting protein structure and inactivating function through displacement of native metal ions (Keyer and Imlay, 1996; Macomber and Imlay, 2009). Organisms have developed complex resistance mechanisms to deal with deleterious copper-induced reactions while satisfying supply for intracellular copper-requiring biological processes.

The copper (Cu) resistance system was initially discovered in *E. coli* and has been widely studied. *Escherichia coli* has evolved two chromosomal encoded *cue* and *cus* systems and a plasmid-encoded *pco* system to resist copper stress (Argüello *et al*., 2013; Bondarczuk and Piotrowska-Seget, 2013; Solioz, 2018). Many copper based bactericides and fungicides have been used in agriculture over a period of time such as Bordeaux mixture, which is the sixth highest selling product in this regard (Cha and Cooksey, 1991). Cu^{2+} was found to be an integral component that impairs protein activity by damaging nucleic acids ultimately, leading to the suppression of microbial activity (Zhang *et al*., 2018). Experimental pieces of evidence also showed that a low concentration of copper ions could effectively protect the plants against bacterial infection by activating defense signaling pathways (Liu *et al*., 2015). For instance, the ethylene (ET) biosynthesis pathway, which is involved in plant immunity, is induced by Cu^{2+} in Arabidopsis (Liu and Zhang, 2004). Cu^{2+} repressed the expression of genes *StABA1* and *StNCED1* for abscisic acid (ABA) biosynthesis, eliciting ET-dependent immunity against bacterial and fungal pathogens (Liu *et al*., 2020). Additionally, copper composites have been used as an effective treatment against bacterial spot disease, as copper composites improve the efficacy of metallic copper by reducing particle aggregation providing a strong shield against bacterial speck (Strayer-Scherer *et al*., 2018).

However, copper resistance has evolved in phytopathogenic bacteria due to extensive use of copper-based bactericides for plant disease control. Since the first description of the copper-inducible system in *Pseudomonas syringae* pv. tomato (Pst) (Cooksey, 1987), many copper resistance systems have been identified in numerous plant-pathogenic species of *Pseudomonas* (Cazorla *et al*., 2002; Gutiérrez-Barranquero *et al*., 2013; Colombi *et al*., 2016), *Xanthomonas* (Lee *et al*., 1994; Behlau *et al*., 2011, 2012, 2013), *Pantoea* (Nischwitz *et al*., 2007) and *Erwinia* (Al-Daoude *et al*., 2009; Águila-Clares *et al*., 2018). Although there are some homologous copper resistance genes between *E. coli* and plant pathogenic bacteria, they differ in gene size, genetic organization and molecular regulation. Thus, plant-pathogenic bacteria have evolved different copper response mechanisms due to diverse living conditions, host stresses, and adopted ecological niches. In the present review, the molecular mechanisms related to copper resistance developed by plant-pathogenic bacteria are summarized, with emphasis on *Pseudomonas* and *Xanthomonas* species. It suggested that some mechanisms are unique in plant pathogenic bacteria, and some occur in *E. coli* and plant pathogens.

COPPER RESISTANCE SYSTEMS IN *PSEUDOMONAS SYRINGAE*

Pathovars of *P. syringae* are plant pathogens that can severely damage their hosts. Long-term utilization of copper compounds for control of these pathogens has resulted in the generation of copper resistant strains, compromising the efficacy of copper (Sundin *et al*., 1989; Zhang *et al*., 2017). In Pst, four copper response genes, namely, *copABCD* are localized within a 35-kb pPT23D plasmid controlled by one promoter which is specifically induced by copper ions (Cooksey, 1987; Cha and Cooksey, 1991). These genes have similarities to corresponding *pco* genes in *E. coli* (Silver and Walderhaug, 1992).

CopA, a 72 kDa periplasmic protein, shares similarity with multicopper oxidase CueO from *E. coli* (Arnesano *et al*., 2002). The particular motifs rich of methionine, histidine, and aspartic acid have enabled CopA protein to bind up to 11 copper ions (Cha and Cooksey, 1991; Cooksey, 1993). Compared with CueO in *E. coli* combined with five copper ions, high copper-binding capacity and oxidase activity give CopA with major role in sequestration and detoxification in copper resistance.

CopB is an outer membrane protein containing numerous methionine residues that can combine copper ions, but the copper binding ability of CopB has not yet been proven (Arnesano *et al*., 2002; Puig *et al*., 2002; Zhang *et al*., 2006).

CopC is a 10.5 kDa soluble molecule with a β-barrel structure. This protein comprises two completely different but interdependent binding sites for, respectively, reduced Cu(I) and oxidized Cu(II). In periplasmic space, copper ions probably substitute two sites due to change in oxidation state (Zhang *et al*., 2006). CopC has been proposed to function as a redox switch to maintain free copper ion concentrations at sub-picomolar levels. When a Cu(II) site is empty, the Cu(I) ion is oxidized by air, but when both binding sites are occupied, no oxidation occurs, showing that CopC acts as a Cu chaperone in oxidizing periplasm, potentially interacting with its neighbor proteins (Zhang *et al*., 2006). A hypotheti-

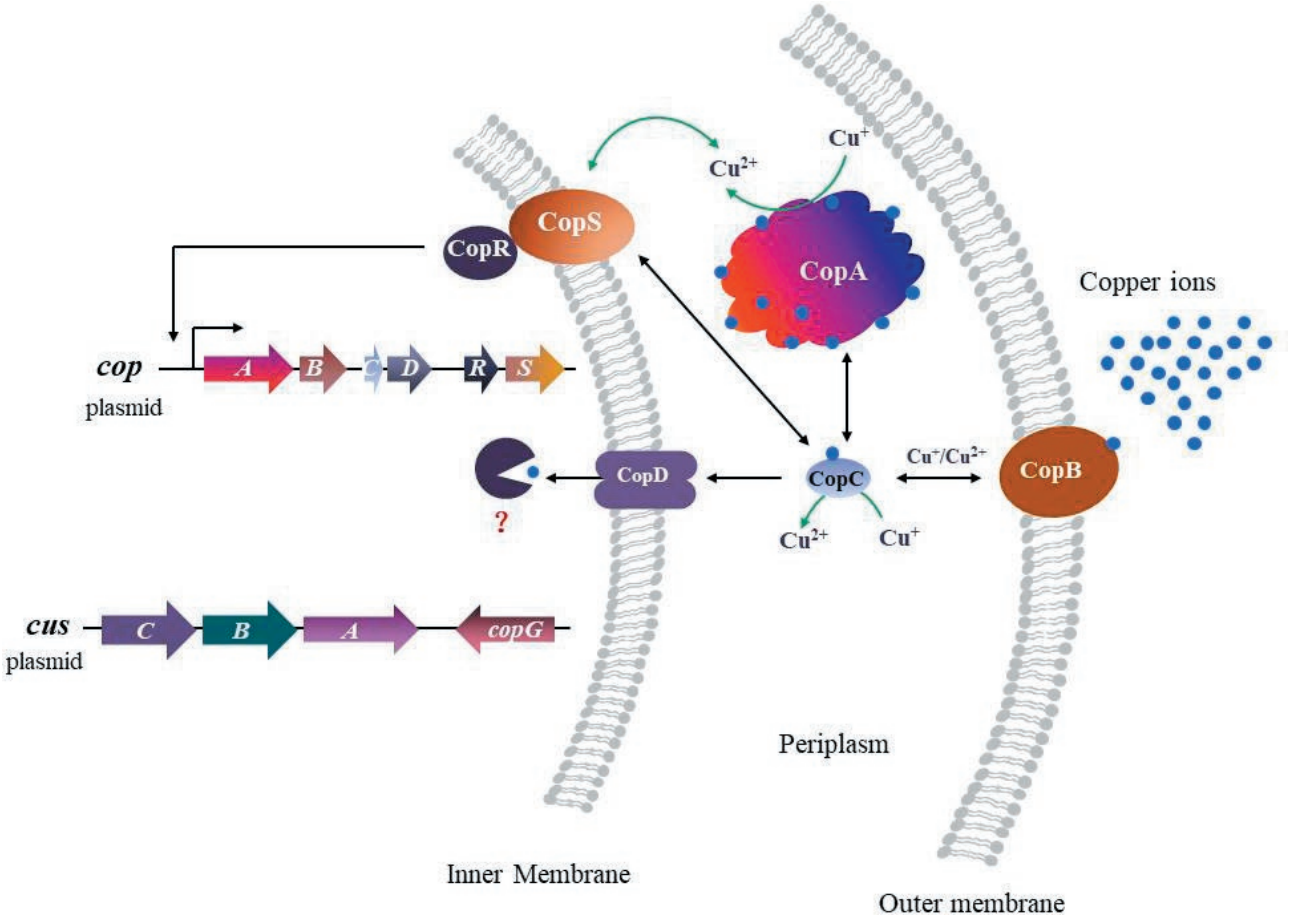

Figure. 1. Proposed model of encoded proteins involved in copper resistance in *Pseudomonas syringae*. Arrows indicate interactions between proteins. CopR induces the expression of *copABCD* via CopS which detects excess periplasmic copper. CopA sequesters excess periplasmic copper due to its strong binding ability; CopB combines with copper; CopC transfers copper from CopA to CopD, and CopD then transports copper into bacterium cytoplasm. CopC also functions as a redox switch to maintain free copper ion concentrations in solution at sub-picomolar levels. The structure of the *cus* operon and *copG* in plasmids of *P. syringae* pv. syringae is a novel efflux system, but the location of these proteins in cell has not been identified.

cal model has been suggested that CopC interacts with CopA and/or CopB to detoxify excess copper (Arnesano *et al.*, 2002) (Figure 1).

CopD is a 33 kDa protein located in plasma membranes, and contains eight predicted transmembranous helices and some conserved histidine residues (Arnesano *et al.*, 2002). CopD transports essential copper ions delivered by CopC through inner cell membranes into the cytoplasm to balance the abundant periplasmic copper sequestered from CopA and CopB (Cooksey, 1993; Arnesano *et al.*, 2002). CopD and CopC are mutually involved in copper uptake in cytoplasm resulting in increased copper accumulation and copper sensitivity (Arnesano *et al.*, 2002).

Except for an induction by high levels of copper ions, transcription of the *copABCD* operon requires a two-component regulatory system CopRS. *CopRS* genes are the downstream components of *copABCD* operon with similar transcriptional orientation but constitutive expression (Mills *et al.*, 1993). CopS acts as a sensor kinase that traverses cytoplasmic membrane and detects copper concentration in the cell periplasm. When the copper ion binds to CopS, a conserved histidine residue is autophosphorylated. Upon phosphorylation of conserved aspartic acid residue, CopR consecutively activates the expression of *copABCD* (Cooksey, 1993; Mills *et al.*, 1993).

Although copper resistance genes in *P. syringae* pv. syringae (Pss) isolated from mango trees can hybridized to *copABCD* DNA, the homologues to *copABCD* are present in a 62-kb plasmid, showing rich diversity (Cazorla *et al.*, 2002). Further studies detected a novel plasmid

structure located between *copABCD* and *copRS* has been detected, that encoded the efflux system *cusCBA* and a putative metal transporting P-type ATPase *copG* in 62-kb plasmid (Gutiérrez-Barranquero *et al.*, 2013). This arrangement has also been observed in other *P. syringae* pathovars affecting different hosts in several countries, and with high sequence similarity (Renick *et al.*, 2008; Studholme *et al.*, 2009; Cai *et al.*, 2011). The genetic organization is involved in the increase of copper resistance in Pss strain (Gutiérrez-Barranquero *et al.*, 2013). In a recent study, a new Tn*7* transposon containing copper resistance genes (COARS Tn*7*-like) has been localized in the chromosome of Pss strains from mango trees. This new COARS Tn*7*-like was found to confer high levels of resistance against copper sulphate that could probably be due to the continuous application of copper. *P. fluorescens* and *Pseudomonas syringae* pv. actinidiae (Psa) also possess the same genomic sequence of COARS Tn*7*-like transposon (Aprile *et al.*, 2020). In addition, the model Pss strain B728a, responsible for brown spot of bean, contains the *copABCD* operon in its chromosome. Pss strain B728a is an epiphyte that feeds on the surface of leaves, from where it can colonize the plant and behave as a pathogen (Vaughn and Gross, 2016).

Zhang *et al.*, (2017) assessed copper resistance in *P. syringae* pv. phaseolicola (Psp) responsible for halo blight disease in beans. Bacterial populations on liquid NB media indicated that 28 out of 35 (80%) strains of this pathogen were resistant to copper, and the bacterial population was similar to that grown on casitone-yeast extract (CYE) agar. Both types of strains have an adequate rate of copper i.e., 161 mg mL^{-1} $CuSO_4$, indicating that CYE agar containing copper can be used for rapid evaluation of copper resistance in this pathogen. Further experiments showed that addition of mancozeb enhanced the effectiveness of copper hydroxide against Psp strain, as mancozeb elevates the solubility of fixed copper.

Psa, causal agent of kiwifruit canker disease, was found to be resistant against copper through integrative conjugative elements (ICEs) and plasmids. Further analyses showed that Psa strains containing genes *czc/cusABC* and *copABCD* were not only resistant to copper but also resistant against arsenic and cadmium. Out of seven strains examined, five showed resistance to copper encoded by ICEs lying at different positions in the Psa genome (Colombi *et al.*, 2017). In general, *P. syringae* pathovars respond to copper stress mainly through sequestration and compartmentalization of the element in cell periplasm and outer membranes (Cha and Cooksey, 1991; Cooksey, 1994). This mechanism is different from the *cue* and *cus* system in *E. coli*, which exhibits resistance by pumping and reducing cellular accumulation of copper (Rensing and Grass, 2003; Bondarczuk and Piotrowska-Seget, 2013). However, the *cus* system in *P. syringae* has an additional efflux mechanism (Gutiérrez-Barranquero *et al.*, 2013). Pathovars of *P. syringae* have evolved a complex response and detoxification system to deal with copper stress in natural environments.

COPPER RESISTANCE SYSTEMS IN *XANTHOMONAS*

Three distinct copper resistance systems have been detected in plant pathogenic—*Xanthomonas*, including a well-known copper-inducible chromosomal *cohABCD* system and a plasmid-born *copLAB* system. The *cusAB/smmD* system similar to that of *Stenotrophomonas maltophilia*, has been discovered from the plasmids of *Xanthomonas* strains, including *X. citri* subsp. *citri*, *X. gardneri,* and *X. euvesicatoria* (Richard *et al.*, 2017). The plasmid-encoded *cop* genes play dominant roles due to the presence of chromosomal copper resistance genes in copper sensitive *Xanthomonas* and *Pseudomonas* strains (Cooksey *et al.*, 1990; Lim and Cooksey, 1993; Behlau *et al.*, 2011). Since copper resistance systems in xanthomonads vary among different species and strains, current understandings of *X. arboricola* pv. juglandis, *X. axonopodis* pv. vesicatoria, and *X. citri* subsp. *citri* is summarized below.

Xanthomonas arboricola *pv.* juglandis

The chromosomal *cohABCD* operon has been fully elucidated in *X. arboricola* pv. juglandis C5, which is the homologous system of *copABCD* in *P. syringae*. CohA protein shares 65% similarity with CopA from Pst, and contains three highly conserved regions of multicopper oxidase. Similarly to CopA, CohA has been proposed to bind four copper ions due to the presence of only one tandem repeat of MX_2MXHX_2M (Lee *et al.*, 1994). Although CohB and CopB share 45% similarity of amino acid sequences, the N terminus of CohB has a hydrophilic region while, CopB contains a hydrophobic region. CohA is a cytosolic protein and CohB has been detected only in cytoplasmic membranes, showing distinctive differences from their homologues in Pst (Teixeira *et al.*, 2008). *CohAB* are essential for copper resistance, while *cohABCD* are required for complete resistance to copper. Inactivation of *cohAB* in other *Xanthomonas* strains supported this conclusion (Teixeira *et al.*, 2008).

The plasmid-borne *copLAB* gene cluster has also been identified from Italian strains of *X. arboricola* pv. juglandis by PCR amplification (Giovanardi *et al.*, 2016).

The sequence of *copA* shares 78% similarity with *cohA* in the chromosomal *cohABCD* operon. In contrast, neither *copL* nor *copB* exhibited sequence similarity with any gene member of the *cohABCD* operon. This variance in copper resistance gene organization within one same species indicated that the genetic basis for copper resistance varies at the intraspecific level.

Xanthomonas axonopodis *pv.* vesicatoria

The *copLAB* resistance system was first detected in the plasmid of *X. axonopodis* pv. vesicatoria 7882. The gene cluster (based on DNA or amino acid sequence) was different from common copper resistance systems in pseudomonads and *E. coli*. CopL, a 122 amino acid protein, exhibited a regulatory role required for *copA* induction under copper stress, since knock-out *copL* resulted in a complete loss of copper-dependent transcription of *copA* (Voloudakis *et al*., 2005). CopL is rich in histidine and cysteine residues that can bind to copper ions. However, expression of *copL* is copper-independent, and is transcribed at the lowest level dominated by a constitutive promoter lacking strong ribosome binding sites (Voloudakis *et al*., 2005). *CopLAB* is widely distributed in the plasmids of *Xanthomonas* from different world regions (Behlau *et al*., 2011; Richard *et al*., 2017), and has also been found on the chromosome of a few *Xanthomonas* strains and *Xylella fastidiosa*, regardless of their copper sensitivity or resistance (Simpson *et al*., 2000; da Silva *et al*., 2002; Potnis *et al*., 2011; Kong *et al*., 2018). *CopL* has been found to be the least conserved *cop* gene in previously sequenced xanthomonads (Behlau *et al*., 2013). However, *copA* is the most conserved copper resistance gene in xanthomonads that have been extensively studied. Several amino acids deletion mutation could cause copper sensitivity (Kong *et al*., 2018). *CopB* is also probably not as important as *copA*, because disruption of *copB* did not result into complete removal of copper resistance (Behlau *et al*., 2011).

Copper resistance in *X. axonopodis* pv. vesicatoria was regarded as only plasmid-born (Bender *et al*., 1990; Garde and Bender, 1991), until a unique chromosomal copper resistance gene cluster was identified in *X. axonopodis* pv. vesicatoria strain XvP26 (Basim *et al*., 2005). Five open reading frames (ORFs), ORF5, ORF4, ORF3, CopR, and CopS are sequentially arranged in this cluster. Total genomic DNA digests of XvP26 could not be hybridized by the *cop* gene cluster in *X. campestris* pv. vesicatoria as indicated using Southern hybridization analysis. CopR, ORF3, and ORF4 are major determinants for complete resistance to copper. CopR contains a conserved palindrome copper box motif, which is essential for copper-inducible activity at the *pcoA* promoters in *E. coli* (Rouch and Brown, 1997). Although defect in the *copS* gene showed no effect on copper resistance, ORF3 failed to respond to copper induction (Basim *et al*., 2005). Integrity of *copS* played an essential role in completing the two-component signal transduction system in *X. campestris* pv. vesicatoria. The CopRS two component regulatory system, has only been found in *X. axonopodis* pv. vesicatoria XvP26, and not in other *Xanthomonas* strains (Basim *et al*., 2005).

Xanthomonas citri *subsp.* citri

A more complicated *copLAB* gene cluster was identified in the *X. citri* subsp. *citri* A44 plasmid (Behlau *et al*., 2011). Compared the *copLAB* operon in *X. arboricola* pv. juglandis and *X. axonopodis* pv. vesicatoria, *copMGCDF* have been identified as the downstream of *copLAB* genes in *X. citri* subsp. *citri* A44. *CopLAB* are the most important genes essential for copper resistance in *X. citri* subsp. *citri* A44, while *copMGCDF* displayed a dose-dependent effect (Behlau *et al*., 2011). CopL possibly regulated *cop* gene expression by interacting with the intergenic region between *copL* and *copA* (Behlau *et al*., 2012). It has been assumed that CopM is a cytochrome c oxidase involved in electron transport, CopG is a hypothetical export protein, CopC and CopD are transmembrane transporter proteins, and CopF is a putative copper-transporting p-type ATPase (Behlau *et al*., 2011). However, elucidation of the specific functions requires more experimental evidence.

In *X. citri* subsp. *citri* strain LM199, the *copLAB* amplicon could not be detected by PCR. The *copABCD* copper resistance system is plasmid-derived, showing more than 97% similarity with the chromosomal *cohABCD* system of *X. arboricola* pv. juglandis (Pereira *et al*., 2015). Although it lacks the two-component regulator *copRS*, a MerR transcriptional regulator, which controls the transcription of proteins CopA and CueO in *E. coli* (Stoyanov *et al*., 2001; Sameach *et al*., 2017), was found close to the *copABCD* cluster (Richard *et al*., 2017). The genetic arrangement and composition of homologues of these plasmidic and chromosomal copper resistance genes in different *Xanthomonas* strains is illustrated in Figure 2.

An HME-RND system *cusAB/smmD* was identified in the plasmid of several *X. citri* subsp. *citri* strains from Réunion, Martinique and Argentina, which showed more than 95% amino acid similarity to RND efflux pumps of *S. maltophilia* isolated from the citrus phyllosphere (Crossman *et al*., 2008). The components of the HME-RND system in *X. citri* subsp. *citri* contain a CusA inner membrane pump, a CusB periplasmic protein

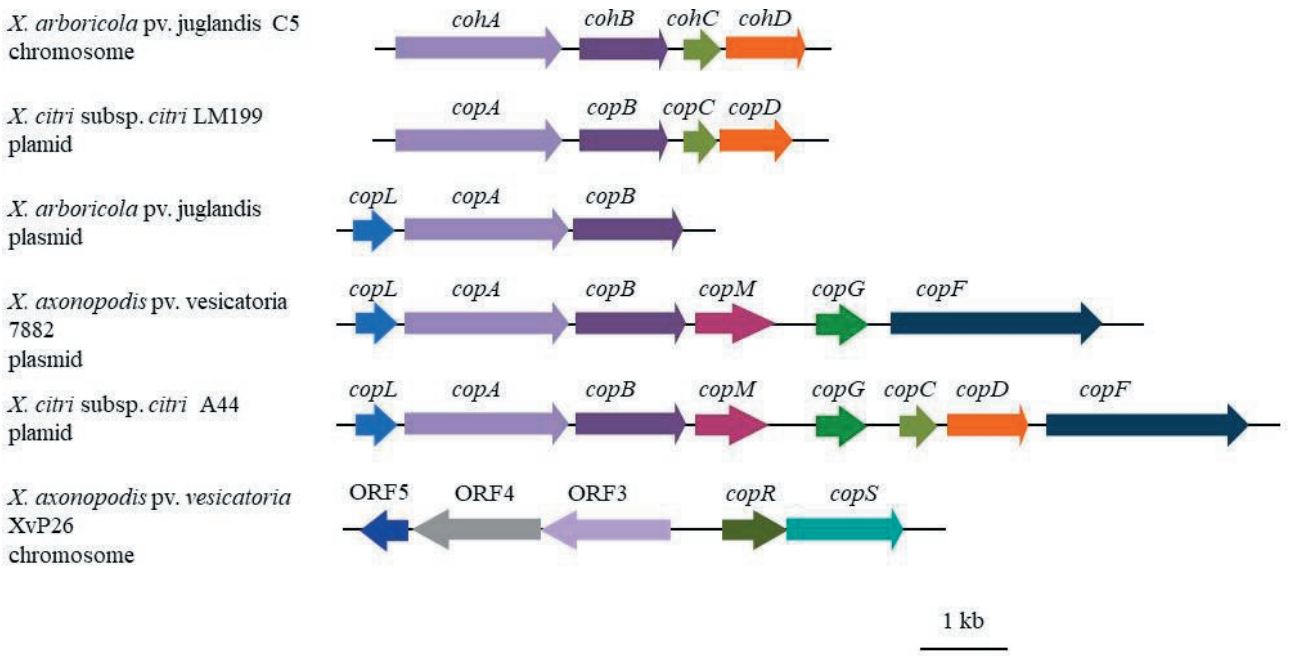

Figure. 2. The genetic arrangement and composition of copper resistance genes in *Xanthomonas* strains. Areas with the same colour indicate homologous genes among the strains. Chromosome indicates the corresponding gene cluster located in the chromosome. Plasmid represents the gene cluster present in a plasmid.

and an SmmD outer membrane protein (Richard *et al.*, 2017). However, *cusAB*/*smmD* is not widely distributed among xanthomonads, and only exists in copper resistance *X. citri* susp. *citri*, *X. gardneri, X. euvesicatoria* and *X. vesicatoria* ATCC 35937 (Richard *et al.*, 2017).

In a Chinese *X. citri* subsp. *citri* strain 29-1, deletion mutation of the conserved membrane protein gene *XAC1347* led to reduced resistance to copper ions (Guo *et al.*, 2015; Fan *et al.*, 2018). *XAC1347* is low in cysteine and methionine residues with no histidine residue, implying that *XAC1347* has little ability to bind copper ions. This indicates this gene may play a role in maintaining cell integrity and osmotic balance (Cybulski and de Mendoza, 2011; Kar *et al.*, 2017). The two-component regulator *colRS* regulates the expression of *XAC1347*, and is involved in copper resistance in *X. citri* subsp. *citri* (Yan and Wang, 2011; Fan *et al.*, 2018). As it is distributed in all known *X. citri* subsp. *citri* strains, this gene could be a universal mechanism required for copper osmotic balance by this pathogen.

ACQUISITION OF COPPER RESISTANCE GENES BY PLANT PATHOGENIC BACTERIA

As a result of continuous application of copper-based chemicals, there has been widespread emergence of copper resistant pathogens (Sundin *et al.*, 1989; Behlau *et al.*, 2013; Colombi *et al.*, 2016). In Florida, nearly 100% of *X. euvesicatoria* and *X. perforans* strains were found to be resistant to copper, due to 50 years of application of copper-based chemicals (Pohronezny *et al.*, 1992). Eighty percent of Psp populations in commercial snap bean fields have become copper resistant (Zhang *et al.*, 2017). In contrast, all *X. campestris* pv. vitians strains causing foliar disease in lettuce were sensitive to copper due to less use of pesticides based on the element (Pernezny *et al.*, 1995). Comprehensive research is required on the origins of copper resistance acquired by plant pathogenic bacteria.

Under selection pressure caused by extensive use of copper-based chemicals, plasmid-born copper resistance genes are responsible for developing copper-resistant bacteria in the field. This can be attributed to horizontal gene transfer (HGT), which usually occurs through plasmids conjugation and bacteriophages transduction (Popa and Dagan, 2011; Achtman, 2012; Sen *et al.*, 2013; Hobman and Crossman, 2015). Comparative genomics and phylogenetic network analyses support the acquisition of copper resistance systems through plasmid incorporation by *X. citri* subsp. *citri* populations (Richard *et al.*, 2017; Gochez *et al.*, 2018). *In vitro* conjugation studies of copper resistance determinants that substituted intra- and inter-specifically within plant-pathogenic bacteria

confirmed this conclusion (Sundin *et al*., 1989; Behlau *et al*., 2012). In addition, copper resistance genes from plasmids of phyllosphere microorganisms can be expressed in *Xanthomonas*, although they have not been found in *Xanthomonas* species. This showed that a broad range of copper resistance gene sources is available for HGT in nature.

In addition to plasmid conjugation, copper resistance genes are possibly acquired through the uptake of integrative conjugative elements (ICEs). In the kiwifruit pathogen *P. syringae* pv. actinidiae, acquisition of Psa NZ45ICE_Cu by a copper sensitive strain Psa NZ13 was detected *in vitro* and *in planta* (Colombi *et al*., 2016). As well, several genomic islands, including genes of plasmid origin, were detected on the chromosome of *X. citri* subsp. *citri* (Gordon *et al*., 2015). This evidence supports the conclusion that HGT is the most important process for copper resistance evolution.

CONCLUSIONS AND PERSPECTIVES

Long-term use of copper-based bactericides has led to the increased populations of copper resistant phytopathogenic bacteria. Detailed studies on structure and function of copper resistance systems may allow rational development of new bactericides that inhibit these systems. However, extensive research is required to achieve this goal. The cytoplasmic copper chaperone responsible for the transportation and detoxification of copper ions has yet to be identified. The present has attempted to encapsulate research progress on copper resistance systems in model bacteria, including *Pseudomonas* and *Xanthomonas*. Although the systems have some similarities with those in *E. coli*, biochemical characteristics and crystal structures of various proteins, and the regulatory networks that control the expression are different in both *Pseudomonas* and *Xanthomonas*.

Formulations of copper complexed with heptagluconic acid induce innate plant immunity, and could be used as an alternative treatment against bacterial attack (González-Hernández *et al*., 2018). Considering the existence of plasmid-borne copper resistance systems, the most effective chemical disease control method could be strict adherence to appropriate dosage and frequency of copper sprays, to reduce the probability of transferring copper resistance genes within and between phytopathogenic bacteria. Use of copper composites against copper tolerant strains can also lessen the chances of bacterial resistance as they do not accumulate in soil or water and exhibit higher antimicrobial activity. Following best cultural practices and incorporating biopesticides in copper composite mixture can minimize the chances of bacterial resistance. Overall, rational studies on evolution of copper resistant phytopathogenic bacteria can lead to design more effective formulations of copper-based chemicals and control strategies that could limit the resistance to copper.

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