Comparative genomic analysis reveals organization, function and evolution of *ars* genes in *Pantoea* spp.

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ABSTRACT: Numerous genes are involved in various strategies to resist toxic arsenic (As). However, the As resistance strategy in genus *Pantoea* is poorly understood. In this study, a comparative genome analysis of 23 *Pantoea* genomes was conducted. Two vertical genetic *arsC*-like genes without any contribution to As resistance were found to exist in the 23 *Pantoea* strains. Besides the two *arsC*-like genes, As resistance gene clusters *arsRBC* or *arsRBCH* were found in 15 *Pantoea* genomes. These *ars* clusters were found to be acquired by horizontal gene transfer (HGT) from sources related to *Franconibacter helveticus*, *Serratia marcescens*, and *Citrobacter freundii*. During the history of evolution, the *ars* clusters were acquired more than once in some species, and were lost in some strains, producing strains without As resistance capability. This study revealed the organization, distribution and the complex evolutionary history of As resistance genes in *Pantoea* spp. The insights gained in this study improved our understanding on the As resistance strategy of *Pantoea* spp. and its roles in the biogeochemical cycling of As.

1 INTRODUCTION

A series of arsenite (As(III)) oxidizing bacteria have been successfully isolated and employed to transform As(III) to arsenate (As(V)) for As remediation, because As(V) is much more strongly adsorbed than As(III). Considering the ubiquitous existence of As(III) oxidizing bacteria in groundwater, we hypothesize that the indigenous As(III) oxidizing bacteria in groundwater should affect the speciation of adsorbed As in filters.

In traditional molecular biology research, As resistance traits are revealed primarily based on the cultivation of a specific strain, and it is impossible to study the As strategy of all strains in a genus. Nevertheless, the genomic sequence of a strain contains nearly all of the genetic information. Therefore, fundamental knowledge such as the phylogenetic, the genetic traits of As resistance and its evolutionary history can be obtained through comparative genomic analysis (Colston *et al.*, 2014).

Pantoea is a genus of Gram-negative, facultative anaerobic bacteria. Currently, the genus contains twenty-six species. In 2013, the strain *Pantoea* sp. IMH was an isolate that reported firstly as the strain having the As resistance capability within *Pantoea* species (Wu *et al.*, 2013). Further, we sequenced the genome of *Pantoea* sp. IMH and found two *ars* clusters (*arsR1B1C1H1* and *arsR2B2C2H2*) co-contributing to its As resistance (Wang *et al.*, 2016). However, the evolutionary history and genetic traits of As resistance in genus *Pantoea* are not fully understood.

Herein, we present the first study of the genetic traits of As resistance in *Pantoea* spp., as well as their

evolutionary history. Two vertically transmitted *arsC*-like genes without any contribution to As resistance were found to exist in the 23 *Pantoea* strains. Besides these two *arsC*-like genes, As resistance gene clusters *arsRBC* or *arsRBCH* were found in 15 *Pantoea* genomes. These *ars* clusters were acquired by horizontal gene transfer (HGT) from sources related to *Franconibacter helveticus*, *Serratia marcescens*, and *Citrobacter freundii*. The insights gained in this study improve our understanding on the complex evolutionary history of As resistance genes and their roles in the biogeochemical cycling of As.

2 METHODS/EXPERIMENTAL

2.1 Comparative genomics

All of the orthologous pairs between *Pantoea* test genomes were identified by Pan Genome Analysis Pipeline (PGAP). The common dataset of shared genes among test strains was defined as their core genome. The total set of genes with test genomes was defined as the pan genome. The set of genes in each strain not shared with other strains was defined as the unique genes.

2.2 Strains, plasmids and culture conditions

E. coli and *Pantoea* strains were grown in LB medium (per liter contains: 10 g tryptone, 5 g yeast and 10 g NaCl) or LB plates (LB medium with w/v 1.5% agar) at 30°C. When appropriate, antibiotics were added at the following concentration: 100 µg mL⁻¹ ampicillin. Resistance to As species was tested by plating serial

dilutions of cultures of each strain onto agar plates containing filtered sodium arsenate (Na_3AsO_4).

2.3 Construction of the recombinant plasmids and *E. coli* strains

A 3.86 kb *Bam*HI-*Xba*I DNA fragment containing the complete *arsI* cluster of *P. stewartii* S301, a 3.43 kb *Bam*HI-*Xba*I DNA fragment containing the complete *ars2* gene cluster of *P. agglomerans* Tx10, a 860 bp *Bam*HI-*Xba*I DNA fragment containing the complete *arsC1*-like gene of *P. stewartii* DC283 and a 942 bp *Bam*HI-*Xba*I DNA fragment containing the complete *arsC2*-like gene of *P. stewartii* DC283 were PCR amplified with primers. The above PCR products were ligated to the *Bam*HI-*Xba*I site of plasmid pUC18, yielding plasmids pUC18-*ars1*, pUC18-*ars2*, pUC18-*arsC1*-like and pUC18-*arsC2*-like. Then the plasmids were transferred to *E. coli* AW3110, yielding the recombinant *E. coli* AW3110-*ars1*, *E. coli* AW3110-*ars2*, *E. coli* AW3110-*arsC1*-like and *E. coli* AW3110-*arsC2*-like strains, respectively.

3 RESULTS AND DISCUSSION

3.1 Distribution and organization of As-related genes in *Pantoea* genomes

The *ars* genes in a genome are prone to group together as *ars* clusters (*arsRBC* and *arsRBCH*). Although comparison of the COG assignments of 23 genomes revealed that the DNA sequences between homologous genes within these *ars* clusters are conserved, some variations exist in DNA sequences, which can be divided into two sub-groups (*ars1* and *ars2*). Unlike the two *ars* clusters in *Pantoea* sp. IMH, only one *ars* cluster, either *ars1* or *ars2*, was observed in other strains. The *ars* gene clusters generally exhibited more than 80% identity within each sub-group and about 54% identity between two sub-groups. Actually, the *ars* clusters were not detected in 8 strains including Sc1, BL1, 9140, DC283, MP7, C91, ND04 and FF5. Moreover, two *arsC*-like genes with only 25% homology (*arsC1*-like and *arsC2*-like) were found in the 23 genomes. Based on the different *ars* genes distributions, the 23 strains were categorized into 4 sub-groups.

3.2 Evolution and the origin of *ars* clusters

The distribution and organization of *ars* genes in *Pantoea* raise a question as to their evolution. We detected the G+C content of *ars* clusters and their corresponding genomes. The results indicated that these *ars* clusters may be acquired in *Pantoea* strains by HGT. To further elucidate the evolution of the *ars* gene clusters, we compared the chromosomal regions flanking the *ars* gene clusters among the 23 *Pantoea* strains and found that the genes in the upstream and downstream regions were conserved among strains of the same species.

To gain insights into the origin of *ars* genes clusters in *Pantoea*, a NJ phylogenetic tree was constructed based on the *ArsRBC* protein sequences. The results imply that the *ars1* cluster may be acquired via HGT from *Franconibacter helveticus*, and *ars2* from *Serratia marcescens* and *Citrobacter freundii* in early evolutionary history.

3.3 Functional analysis of *ars* gene and *arsC*-like genes

The growth of the yielded recombinant *E. coli* strains *E. coli*-*ars1* and *E. coli*-*ars2*, was tested in 5 mM concentration As(V). Both *E. coli*-*ars1* and *E. coli*-*ars2* survived in 5 mM As(V), and *E. coli*-*ars1* grew better than *E. coli*-*ars2*. This result suggests that both the *ars1* and *ars2* clusters enabled *E. coli* AW3110 to resist As, and *ars1* seemed to have a more effective As resistance capability than *ars2*. Neither the *arsC1*-like nor *arsC2*-like gene enables *E. coli* AW3110 to resist As. In line with the alignment result, the function analysis demonstrates that these two *arsC*-like genes do not contribute to As resistance.

4 CONCLUSIONS

Arsenic resistance gene clusters *arsRBC* or *arsRBCH* were found in most of *Pantoea* genomes. These *ars* clusters were found to be acquired by horizontal gene transfer (HGT) from sources related to *Franconibacter helveticus*, *Serratia marcescens*, and *Citrobacter freundii*. During the history of evolution, the *ars* clusters were acquired more than once in some species, and were lost in some strains, producing strains without As resistance capability. Our results improved the understanding of the As resistance strategy in *Pantoea* spp.

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