



Plasmid pMOL30 (234 kb) has a large backbone (B) in figure) with many orthologs of pBVIE01 of *Burkholderia vietnamiensis* and carries the genes involved in the resistance to cobalt, zinc, cadmium, lead and mercury, which are located on one region called CMGI-30a, while the genes involved in the resistance to copper and silver are located on a second region CMGI-30b. A general characteristic observed for both plasmids is that genomic islands carrying the genes involved in heavy metal resistance are flanked by small MGEs on a general plasmid backbone.

Expression analysis of pMOL28 and pMOL30 under various metal conditions indicated that the genes encoding resistance to a particular heavy metal are also induced by various other heavy metals. Therefore, it appears that the response of CH34 to heavy metals, which in

contaminated environments are generally mixtures of multiple metals, consists of an initial upregulation of all metal resistance genes that could be followed by a more substrate-specific response. In addition, novel genes were found to be induced by heavy metals which encode for glycosyltransferases involved in cell wall biosynthesis and probably could act as maintainers of membrane integrity under heavy metal attack.

Besides pMOL28 and pMOL30, a wide variety of other MGEs have been identified namely 13 Insertion Sequences (IS) elements and 5 transposons that can move around to different positions within the genome, and at least 11 genomic islands. A few of these MGEs will be highlighted. A genomic island identical to one found in *P. aeruginosa* clone C contains a P type ATPase (CadA) involved in Zn(II), Cd(II) and Pb(II) resistance and *mer* genes. Two additional genomic islands belonging to the family of catabolic transposon Tn4371 of *C. oxalatica* were found carrying genes involved in carbon dioxide fixation and in hydrogen reduction. Particularly, interesting was the discovery of two transposons, TnCme2 and TnCme3, which do not carry any selectable marker, therefore underlining the power of synteny for the discovery of MGEs with novel functional associations. The transposon TnCme2 was found in 7 copies in the genome of CH34 and contained next to the typical genes related to transposition one single gene encoding for protein involved in type II secretion. Transposon TnCme3 carried genes encoding for a sulphate permease, a universal stress protein and a DnaK suppressor protein.

The genome sequence of *C. metallidurans* CH34 showed a wide variety of genetic traits probably acquired through horizontal transfer over time. These Mobile Genetic Elements (MGEs) played a central role in the genetic evolution and diversification of the bacterium CH34 and its adaptation to environmental or man-made changes. This work allows a new insight in the (up to now unnoticed) diversity of MGEs what will be of importance for our current and future biosafety studies linked to the monitoring and prevention of gene dissemination in confined environments (space research, planetary stations).

Future work

The regulation and organization of the genes involved in the resistance to heavy metals will be further analyzed. This genetic information will further unravel its adaptation capabilities and will render additional insights for potential experiments studying the interaction of CH34 with oxidative agents, radiation and radionuclides. A sound understanding of the global response of *C. metallidurans* CH34 to heavy metals (or other agents) allows further development of its biotechnological applications in e.g. bioremediation.

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