Design of a direct positive selection method to identify genes involved in metals-efflux systems regulation

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ABSTRACT

While Cu and Zn are essential nutrients for Bacillus subtilis, Cd is toxic per se. However, in high concentrations such metals will be a major threat to the cell. Different bacteria express metal efflux systems as response to the increase of the intracellular concentration of a given metal. CPx-type P-type ATPases are the most common efflux systems used by bacteria to excrete toxic metals. B. subtilis genome revealed the presence of three CPx type ATPases, zosA, yvgW and yvgX. ZosA encodes a peroxide induced zinc uptake system and it is under the control of the peroxide response repressor, PerR. While, yvgW and yvgX encode CadA; and CopA; a Cd, Zn and Co and Cu efflux systems respectively. In several bacteria, the metal efflux systems are under regulation of proteins from the MerR or ArsR families. Here the design and application of a method to directly select for mutations in genes that regulate metal efflux system are reported. This method employs the modification of the zinc uptake repressor (zur) gene promoter to be solely expressed under the control of a given metal efflux system promoter. This repressor is tightly suppresses the expression of yciC, zinc uptake gene, promoter fused to the ORFs, encoding a chloramphenicol resistance and -galactosidase enzyme (P-yciC-Cat-lacZ). Upon addition of metal, the modified Zur protein is expressed leading to the full repression of P-yciC-Cat-lacZ, which cause the cell to be sensitive to chloramphenicol. Upon random transposon mutagenesis, mutants that no longer activate the expression of zur were isolated directly by selecting for chloramphenicol resistance clones. The method was tested using Cd(II)/Zn(II)/Co(II) and copper efflux systems promoters. This method allows direct selection of regulatory mutant without the need for screening of large mutation banks.

Key words: *Identification, genes, metals efflux, regulation.*

INTRODUCTION

Cellular responses to metal starvation are complex given the toxicity of such nutrients in high concentrations. Metals play an essential role in bacterial metabolism. Some metals function as catalysts for biological reactions, stabilizers of protein structures and serve in maintaining osmotic balance. At high concentrations, however, metal ions form unspecific complex compounds, which leads to toxic effects. Bacteria have adapted to metals through a variety of chromosomal-, transposon- and plasmid-encoded resistance systems, for reviews, (Bruins *et al.*, 2000; Nies, 1999;