

CONSTRUCTION OF BROAD HOST RANGE ARSENIC SENSOR PLASMIDS

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Arsenic contamination in ground water is a serious problem in the world. A simple and sensitive arsenite sensor system is needed. At this work we pretend to constructed broad host range arsenic sensor plasmids. The *ars* operon of *Escherichia coli* and *Thiobacillus ferrooxidans* consists of a set of structural genes and a regulatory gene, *arsR*. The ArsR protein is a repressor that binds the promoter region of the *ars* operon and prevents transcription of the downstream genes. In the presence of arsenite, Ars R binds with arsenite and dissociation from the promoter region. Initially we constructed two broad host range plasmids, pBB-Lac and pBB-GFP, which consists of the pBBR1MCS vector with promoterless reporter genes cloned, the first with betagalactosidase gene (*lacZ*) of *Escherichia coli* and the second with the green fluorescent protein gene of *Aequorea Victoria*. After that the *ars* operon promoter region and the gene that codified for the regulatory protein (*arsR*) were amplified and cloned upstream the reporter genes. Since the promoter and operator site for ArsR-dependent gene expression are upstream of *arsR* itself, a basal level of *arsR* expression is required for the system to function. For this reason, gene fusions placed downstream of *arsR* will be subject to considerable background expression of the reporter gene. To have a system that will be more sensitive to low level of arsenite we introduced a second binding site for ArsR (operon *ars* promoter region) downstream the gene *ars R* and upstream the genes reporters.

Key words: arsenic, promoter, sensor