

Role of BcrAB transporter and undecaprenol kinase in bacitracin resistance and cross-resistance to other antibiotics

The recently described operon *bcrABD*, codes for two different mechanisms of bacitracin resistance in *Enterococcus*. This operon is described to be regulated by a transmembrane protein, BcrR, which is also binding to the promoter region of *bcrA* and induces the expression of the *bcr* operon in the presence of bacitracin, which turns into a high-level bacitracin resistant (HLBR) phenotype. However, we have recently described strains lacking *bcrR* and *bcrD* genes and still showing an HLBR phenotype. On the other hand, some mutation in the *bcrAB* genes were detected in strains carrying these genes but showing low-level bacitracin resistance. Moreover, both the *bcrABDR* and the *bcrAB* genotypes are disseminated in different environments, as well as the HLBR phenotype, which is surprising because bacitracin has not been heavily used in Europe in the last decade. Therefore, the proposed project has the following objectives and strategies:

- unravel the role of the mutations in the *bcrAB* genes in the low-level bacitracin resistance and of *bcrD* gene;
- understand the reason why *bcrABDR* is only detected in the clinical settings, whereas *bcrAB* is found in all environments where HLBR is detected in *Enterococcus* spp.;
- understand why bacitracin resistance is being selected despite not being used.

In order to do this, we propose the following:

- sequence the *bcrAB* genes of several isolates with different bacitracin resistance phenotypes;
- construct mutants that will confirm the role of the mutations in the bacitracin resistance phenotype;
- sequence the upstream region of *bcrA* in strains carrying *bcrR* and strains not carrying *bcrR*. This will allow us to understand if the two genotypes have evolved separately or from each other;
- study the role of inhibitors of ABC-type of transporters in bacitracin resistance and cross-resistance, in order to know if the BcrAB transporter is also able to transport other antibiotics.
- Construct deletion mutants in the *bcrD* gene in order to understand the role of this gene in bacitracin resistance.

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