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Antimicrobial resistance in Bacilli, transfer and detection

Objectives The current information gap as to the mechanisms of antimicrobial resistance and transfer in *Bacillus* species precludes full understanding and impedes solutions. Molecular biological investigations of resistance and transfer should allow development of a system for rapid and direct detection of antibiotic resistant genes in pathogenic bacteria such as *Bacillus anthracis*.

Conclusions A novel microarray-based hybridisation system was developed for the detection of antibiotic resistance genes in gram-positive bacteria(*) and was optimised, in particular, for highly pathogenic bacteria. This technology was tested with wild strains of *B. anthracis*. *B. anthracis* causing animal death in Africa were shown to be susceptible to the major class of antibiotics and to be free of known transmissible antibiotic resistance genes. It has been shown that *B. anthracis* (using avirulent strains) is able to acquire antibiotic resistance genes from other bacteria by conjugation (as demonstrated and detected with our microarray assay). Additionally, other *Bacillus* of the *cereus* group from different environmental sources were found to harbour tetracycline resistance genes. Among them, a new tetracycline resistance mechanism was found to be localized on a conjugative plasmid. Moreover, in the presence of subinhibitory concentrations of tetracycline and ciprofloxacin, *Bacillus thuringiensis* can develop resistance to these antibiotics.

(*) This microarray has stimulated additional research projects, i.e. at the Laboratory of Food and Biotechnology, Institute of Food Science and Nutrition, ETH Zurich (Prof. Dr. L. Meile) for the detection of antibiotic resistance genes in bacteria from food (Kastner et al. *Syst Appl Microbiol.* 2006 Mar;29(2): 145-55) and at the Institute of Veterinary Medicine, Bacterial Epidemiology and Infectiology, University of Bern (PD Dr. V. Perreten) for the detection of antibiotic resistance genes in *Staphylococcus sp.* from horses (Schnellmann et al. *J Clin Microbiol.* 2006 Dec;44(12): 4444-54).

Main results and findings

Development of a microchip-based hybridization system (ArrayTube) to detect antibiotic resistance genes in gram-positive bacteria including *B. anthracis* The 1st generation ArrayTube was characterised by:

- Containing the majority of the antibiotic resistance genes known to be transferable among gram-positive bacteria (up to 90 resistance genes).
- It has been tested with a large variety of stains harbouring antibiotic resistance genes of *Enterococcus faecalis, Enterococcus faecium, Lactococcus lactis,* avirulent *Bacillus anthracis*. Field studies included *Staphylococcus haemolyticus* and *Clostridium perfringens*.
- A safe labelling protocol was developed for safe application with highly pathogenic bacteria such as *Bacillus anthracis*. This safe protocol has been adopted by other groups of the laboratory that use microarrays.
- The ArrayTube was used for screening *Bacillus anthracis* field strains from Chad for the presence of antibiotic resistance gene. None were shown to harbour a known antibiotic resistance gene.

The 2nd generation ArrayTube was a further optimised version and allowed the detection of 10 additional resistance genes.

Analysis of the antibiotic resistance profile of *Bacillus* of the *cereus* group in Switzerland 145 strains from animal environment, 72 strains from food, 44 of human origin and 3 from water were characterised. The results can be summarised as follows:

- 8.9% of the strains were tetracycline resistant. Intrinsic resistance to penicillin was found in 96.1% of the strains, and 73% of the strains were resistant to the combination amoxicillin-clavulanic acid.
- The 19 tetracycline-resistant strains were tested for the presence of the tetracycline resistance determinants *tet*(L), *tet*(K), *tet*(M), *tet*(S) and *tet*(O). Two stains harboured the *tet*(L) gene on a plasmid, but in the other strains no known genes were found, suggesting a new tetracycline resistance mechanism in *Bacillus* of the *cereus* group.

- In conjugation attempts between the 2 strains of *B. cereus* harbouring a *tet*(L)-carrying plasmid and *B. thuringiensis,* only one of the *tet*(L) plasmid was mobilized into *B. thuringiensis*.
- In conjugation attempts with 4 tetracycline resistant strains that did not carry any known tetracycline determinant, a 56-kb large plasmid, named pLVP1401, was transferred from *B. cereus* into *B. thuringiensis.* Characterisation of the plasmid pLVP1401 revealed that it is a completely new DNA molecule of 56242 bp and that it does not carry any genes showing similarities with known tetracycline resistance genes. Four genes, each coding for a putative membrane protein, were found. They might play a role in efflux of tetracycline.
- In conjugation attempts between *B. cereus* and *B. anthracis,* no transconjugants were obtained.

Transfer and detection of resistance genes in avirulent strains of *B. anthracis* The results of conjugal transfer assays of antibiotic resistance genes between enterocci and 3 avirulent strains of *B. anthracis (B. anthracis* 4230 lacking the virulence plasmid pXO1 and containing a deletion of the capsule genes on pXO2, *B. anthracis* BANT2 lacking the capsule plasmid pXO2 and containing a deletion of the virulence genes on plasmid pXO1 and *B. anthracis* lacking the edema factor gene pXO1 and the capsule genes on pXO2) can be summarised as follows:

- The multidrug-resistant plasmid pRE25 (harbouring the 50 kb conjugative plasmid pRE25, which carries 5 resistance genes including *erm*(B), cat_{pIP501}, *aph(3')-III, sat4* and *aadK)* (Schwarz et al. *Plasmid.* 2001 Nov;46(3):170-87) was transferred from *E. faecalis* only into *B. anthracis* 4230 and at low frequency, but not into the other two avirulent *B. anthracis* strains, probably because of incompatibility with the virulence plasmid pXO1.
- Plasmid pRE25 in *E. faecalis* confers resistance to the aminoglycoside antibiotics kanamycin and streptomycin, but when present *in B. anthracis* 4230 it did not confer any resistance to either kanamycin or streptomycin, although the gene *aph(3')-III* could be detected by DNA hybridisation.
- Plasmid pRE39 (harbouring an *erm*(B)-carrying conjugative plasmid) (Teuber et al. *Lebensmittel-Technologie.* 1996;29:182-99) was transferred from *Enterococcus species* into all 3 avirulent *B. anthracis* strains at low frequency.

Production of fluoroquinolones and tetracycline resistant mutants using *Bacillus thuringiensis* as a model *Bacillus thuringiensis* was used as a model to characterise the ability of *Bacillus* of the *cereus* group to acquire resistance when exposed to subinhibitory concentrations of ciprofloxacin and tetracycline. Both tetracycline and ciprofloxacin resistant mutants were obtained.

Publications of the NRP 49 project

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