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J. Frey

Antibiotic resistance genes in food: Molecular identification and transfer between micro-organisms (ABRFOOD)

Objectives In order to produce foodstuffs containing low levels of antibiotic resistant bacteria, broad analysis of the antibiotic resistance gene flow in the environment is required, so that measures to increase food safety by elimination or reduction of resistant bacteria can be taken.

Conclusions Ready-to-eat food (i.e. cheese and meat) as well as starter and probiotic cultures are antibiotic resistance genes reservoirs, and a broad range transfer of antibiotic resistance genes *in vitro* could be demonstrated. Therefore, a new quantitative real-time PCR technique to directly and quantitatively detect enterococci resistance genes (*tet*(M) and *erm*(B)) in ready-to eat food was established. This technique can be potentially applied to detect the copy number of *tet*(M) and *erm*(B) in any environment (i.e. colon). For the routine assessment of about 100 antibiotic resistance genes in starter and probiotic cultures, a new microchip assay was developed and validated in collaboration with Prof. Dr. J. Frey.

Main results and findings

Analysis of ready-to-eat food Independent of the manner of production (organically or conventionally produced), ca. 40% of the analysed cheeses were contaminated with erythromycin resistant enterococci and ca. 60% with tetracycline resistant enterococci. These values are higher than expected by Federal Veterinary Office. On the other hand, no vancomycin resistance was found. In raw milk cheeses, the amount of resistant enterococci, including high level gentamycin resistance, was two-fold higher than in pasteurised milk cheeses.

J. Frey

Analysis of starter and probiotic cultures Starter and probiotic cultures were tested for the presence of about 100 antibiotic resistance genes using a novel microarray assay, developed in collaboration with Prof. Dr. J. Frey. Tetracycline resistance detected in some staphylococci used as meat starters could be ascribed to the gene *tet*(K), whereas the tetracycline resistance of the probiotic bacteria *Bifidobacterium lactis* and *Lactobacillus reuteri* SD2112 was due to the gene *tet*(W). Therefore, surprisingly, starter and probiotic cultures were found to be antibiotic resistance genes reservoirs. The microarray proved to be a highly successful way to assay, within a short time, a lot of samples from the Swiss market.

Characterisation of antibiotic resistance genes in Enterococcus A quantitative PCR technique to directly and quantitatively detect (copy number) enterococci *tet*(M) and *erm*(B) in ready-to eat food as well as in other milieus (i.e. colon) was developed and validated. With this method, 37 different cheeses and 6 different ready-to-eat meat products were analysed, and 282 presumptive antibiotic resistant *Enterococcus* strains were isolated. *Enterococcus faecalis* was found to be the dominant species in cheese, whereas *Enterococcus faecium* was the dominant species in meat. The genotypic characterisation of the resistance genes revealed that:

- all tetracycline ribosomal protection gene (*tet*(M)) positive isolates were positive also for *int* (transposon integrase gene of the Tn916/Tn1545 family).
- all tetracycline efflux resistance gene (*tet*(L)) positive isolates were positive also for *tet*(M) and *int.*
- the erythromycin ribosomal protection gene positive (*erm*(B)) isolates never carried the *mef*(A) erythromycin efflux gene.
- the high level gentamycin resistance in *Enterococcus* isolates from conventionally produced cheese was mediated by the *aac(6')-Ie-aph(2")-Ia.* These isolates also contained the genes *erm*(B), *tet*(M) and *int.* However, the detection of this gentamycin resistance is rare among food related enterococci.

Transfer capacity (in vitro) to narrow and broad host range A new approach to conjugation by co-immobilisation of donor and recipient cells in gel-beads prior to incubation in a fermentation system was developed. This new method allowed higher transfer rates, and it was shown that:

- there is a transfer potential of resistance genes both within enterococci and to other gram-positive recipients (*L. lactis ssp. lactis biovar diacetylactis Bu2-60, L. mesenteroides M7-1, S. aureus 80CR5, L. innocua L19*).
- the gentamycin resistance gene (*aac(6')-Ie-aph(2")-Ia*) is located on a transmissible plasmid.

Publications of the NRP 49 project

Kastner S, Perreten V, Bleuler H, Hugenschmidt G, Lacroix C, Meile L.
Antibiotic susceptibility patterns and resistance genes of starter cultures and probiotic bacteria used in food.
Syst Appl Microbiol. 2006 Mar;29(2):145-55. Epub 2005 Aug 18.

Meile L, Teuber M.
Antibiotika und Resistenzbildung.
In: Eichholzer M, Camenzind-Frey E, Matzke A, Amadò R, Ballmer PE, editors. Fünfter Schweizerischer Ernährungsbericht. Bern: Bundesamt für *Gesundheit;* 2006. p. 403-18.

Meile L, Niederer B, Baumann A, Miescher Schenninger S.
Mikroorganismen als Lebensmittelzusätze: Starterkulturen, Schutzkulturen und Probiotika.
Mitteilungen in Lebensmitteluntersuchungen und Hygiene. 2005;96(1):3-14.

Leisibach S.
Antibiotic resistance genes in food: molecular identification and transfer between microorganisms with emphasis on enterococci
[dissertation No. 15666]. Zurich: Swiss Federal Institute of Technology (ETH); 2004.

Egli T, Koster W, Meile L.
Pathogenic microbes in water and food: changes and challenges.
FEMS Microbiol Rev. 2002 Jun;26(2):111-2.

back to the NRP 49 final report