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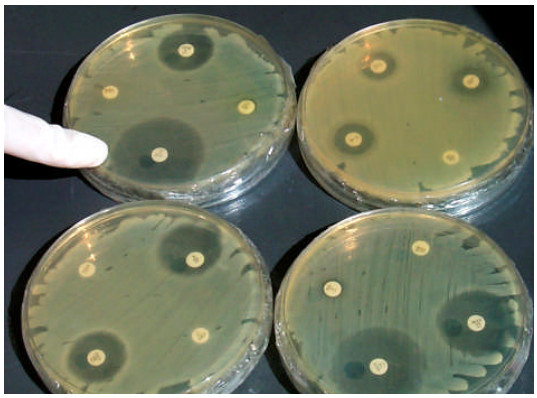
Transfer of antimicrobial resistance from lactic acid bacteria (LAB) to other LAB and to pathogenic bacteria



Key external stakeholders:
Dairy industry, scientists, regulatory personnel, medical doctors, veterinarians, epidemiologists, microbiologists, consumers, EFSA

Practical implications for stakeholders:
Commercial LAB should be examined for antibiotic resistance encoding genes and assessed for transfer to other bacteria.

Main results:

Antibiotic resistance genes were readily transferred between different LAB strains *in vitro*, in the plant model system and to a lesser extent in the rumen model. Transfer to *Listeria* spp. was also observed *in vitro* and in a food system but not to *Salmonella* spp., *S. aureus* or *E. coli*.

Opportunity / Benefit:

This project highlights the importance of strain selection in food fermentations and provides data for a risk assessment of the role of LAB in the emergence of MDR pathogenic bacteria.

Collaborating Institutions:

University College Dublin

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External collaborators: Prof. Seamus Fanning (University College Dublin)

1. Project background:

The emergence and spread of antibiotic resistance encoding genes among bacterial has lead to the development of multiple resistant strains (MDR). These represent a serious public health risk, especially to the immune compromised. LAB have been used in food fermentations for many centuries. However, very little research has been done on their potential role in the emergence of MDR pathogens. In this project the transfer (transconjugation) of antibiotic resistance encoding genes between different LAB and from LAB to pathogenic bacteria was examined *in vitro* (filter and plate mating), in a food matrix and in rumen and plant model systems.

2. Questions addressed by the project:

- Can LAB transfer antibiotic resistance to other strains *in vitro* and in model rumen and plant systems?
- Can LAB transfer antibiotic resistance to pathogenic bacteria *in vitro* and in model food systems?

3. The experimental studies:

Study 1: The ability of three wild-type dairy isolates of lactic acid bacteria (LAB) and one *Lactococcus lactis* control strain to transfer antibiotic resistance determinants to two LAB recipients was investigated using both *in vitro* methods and *in vivo* models. *In vitro* transfer experiments were carried out with the donors and recipients using the filter mating method. *In vivo* mating examined transfer in two natural environments, a rumen model and an alfalfa sprout model. All transconjugants were confirmed by Etest, PCR, pulsed-field gel electrophoresis, and Southern blotting.

Study 2: The transferability of antimicrobial resistance from LAB to *Listeria* spp., *Salmonella* spp., *Staphylococcus aureus*, and *E. coli* was investigated using *in vitro* methods and in a food matrix. Five LAB donors, containing either erythromycin or tetracycline resistance markers on transferable elements were conjugally mated with the pathogenic strains using both the filter and plate mating methods. Transfer to the pathogenic bacteria was also examined in a food matrix consisting of fermented whole milk (fermented with the LAB donors) with the pathogenic recipients added as contaminants during the production process. All transconjugants were confirmed by phenotypic and molecular methods.

4. Main results:

In study 1, high transfer frequencies were detected between LAB using filter mating and in the plant model. Transfer of antibiotic resistance determinants also occurred in the rumen but to a lesser extent. In study 2, antibiotic resistance was readily transferred from the LAB strains to *Listeria* spp. using the filter and plate mating techniques but not in the fermented milk matrix. Transfer to *Salmonella* spp., *S. aureus* or *E. coli* was not detected.

5. Opportunity/Benefit:

LAB are a potential source of resistance determinants that may be disseminated between LAB and to pathogenic strains including *Listeria* spp. This research demonstrated transconjugation in the natural environment and suggests that food matrices such as fermented milk may provide a suitable environment to support gene exchange. Strain selection for fermented foods should also include an assessment of the potential for antibiotic resistance dissemination in a public health context.

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6. Dissemination:

Dissemination was primarily achieved through peer reviewed publication and presentation at national and international conferences.

Main publications:

Morelli, L. Mättö, J., Wilcks, A., Aarts, H. & Bolton, D. 2007. Assessment and critical evaluation of antibiotic resistance transferability in food chain: the ACE-ART project, *International Journal of Science and Marketing for Nutraceutical Actives, Raw Materials and Finished Products* 9, 2-3, 89-92.

Francis, A., A. Meally, D. J. Bolton, C. Gahan, P. D. Cotter, C. Hill and D. O'Beirne 2007. The glutamate decarboxylase acid resistance mechanism affects survival of *Listeria monocytogenes* LO28 in modified atmosphere-packaged foods. Journal of Applied Microbiology, 103, 2316-2324.

Lampkowska, J., Feld, L., Monaghan, A., Toomey, N., Schjørring, S., Jacobsen, B., van der Voet, Sigrid H., Andersen, R., Bolton, D., Aarts, H.,. Krogfelt, K., Wilcks, A. and Bardowski, J. (2008) A standardized conjugation protocol to asses antibiotic resistance transfer between lactococcal species. International Journal of Food Microbiology. 127 (12) 172-175.

Toomey, N., Monaghan, A., Fanning, S. and Bolton, D. J. (2009) Assessment of horizontal gene transfer in Lactic acid bacteria - a comparison of mating techniques with a view to optimising conjugation conditions. Journal of Microbiological Methods, 77 (1) 23-28.

Toomey, N., Monaghan, A., Fanning, S. and Bolton, D. J. (2009) Transfer of antibiotic resistance marker genes between Lactic Acid Bacteria in model rumen and plant environments. Applied and Environmental Microbiology, 75 (10), 3146-3152.

Toomey, N., Monaghan, A., Fanning, S. and Bolton, D. J. (2009) Assessment of antibiotic resistance transfer between lactic acid bacteria and potential foodborne pathogens, using in vitro methods and mating in a food matrix. Foodborne Pathogens and Disease. 6 (8), 925-933.

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