The potential of the breakdown products of casein by *Lactococcus lactis* strain 146 as inhibitory therapeutic agents for MRSA

***Mohammed Al-Mahrous*[1], *Mohammed Alqamber*[2], *James Burnie*[1], *Mathew Upton*[1], *and John Tagg*[2]**
[1]*Medical Microbiology, University of Manchester, Manchester Royal Infirmary, Manchester, UK.*
[2]*Department of Microbiology, University of Otago, Dunedin, New Zealand.*

Background:
lactococci possess a proteolytic system that can release free amino acids, peptides and oligopeptides from casein (milk protein) (Laan & Konings, 1989; Reid *et al.*, 1991; Tan *et al.*, 1992). The proteolysis involves the action of cell wall-associated peptidases (CWAP) (Hugenholtz *et al.*, 1987; Laan & Konings, 1989; Monnet *et al.*, 1987) and subsequent hydrolysis is carried out by several enzymes found in the cell envelope (Tan *et al.*, 1992); which can eventually be taken up by the bacteria (Smid *et al.*, 1989). This fermentation process results in milk diaries flavour and proteinacious end-products. The objective of the current study was to investigate the end-products of casein degradation by *Lactococcus lactis* strain 146, as inhibitory agents for MRSA.

Methods:
- Investigation of the end-products of *L. lactis* strain 146 was performed using plate-diffusion method from casein-containing minimum essential media (MEM).
- Casein-free MEM was used as a negative control for the inhibitory end-products, on which alternative growth factors were included.
- Purification and/or concentration of the end-products in broth supernatants was carried out using ammonium-sulphate precipitation, XAD-2 resin separation, cation-exchange; then C18 reverse-phase chromatography.
- Partial purification of non cell-associated (free) inhibitors in broth supernatants was carried out using methanol precipitation.
- Sep-Pak® cartridge was incorporated to further separate contaminants.
- Further purified using cation-exchange column chromatography (ProPac™).
- High-pressure liquid chromatography was the last step in the purification stage.
- Speed-Vac® was used to concentrate methanol fractions.
- MALDI TOF/TOF was used for molecular mass determination.
- All biological tasting of the active fractions were carried out using well-diffusion assay (du Toit & Rautenbach, 2000).
- A range of published and/or designed primers was used for PCR of gene(s) responsible for the synthesis of CWAP in strain 146.
- The gene was cloned using T-tailed vector; and then sequenced.

Results:
Supernatant from casein-containing media displayed activity against MRSA, but not casein-free media (Figure 1). The reversed-phase HPLC profile of the processed active fractions revealed several peptide species (Figure 2). Determining the mass of peptides with MS showed that they were seized in a window of 0.9 and 5 kD (Figure 3). Among the tested primers, BG95 / 146CEP–invlwoer1; BG97 / 146CEP–invlwoer1; BG95 / 4CA showed positivity with strain 146 on PCR. However, cloning was successful; the sequence data of the vector still needs further analysis.

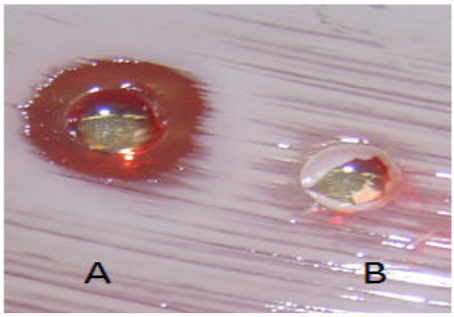

Figure 1: A is the inhibitory activity of supernatant for the growth of strain 146 in casein-containing minimum essential medium (MEM) against epidemic MRSA-15; whereas, B is the negative control for the lack of inhibitory activity of the supernatant for the growth of strain 146 in casein-free MEM.

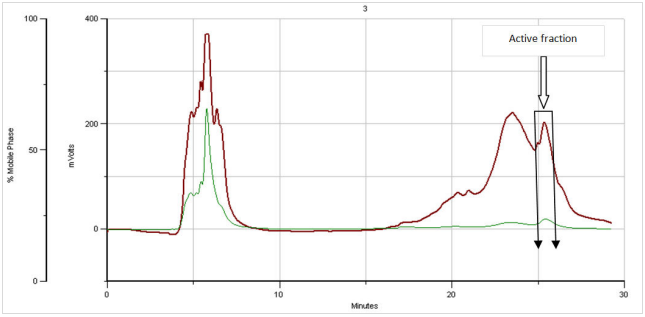

Figure 2: Active fraction, from growth of *Lactococcus lactis* strain 146 in minimum inhibition media (MEM) + casein, using reverse-phase liquid chromatography.

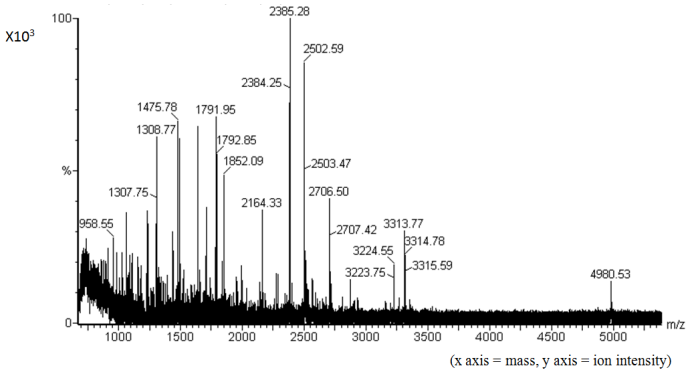

Figure 3: Detection of peptide species of casein breakdown products by *Lactococcus lactis* strain 146 using mass spectrometry. Resulting amino acids were detected on a time-of-flight (TOF) mass spectrometer with matrix-assisted-laser-desorption ionization (MALDI).

Conclusions:

The effect of the breakdown-products of casein by *L. lactis* strain 146 against MRSA suggests the potency of these peptides as future therapeutic agents for treating the highly drug-resistant *Staphylococcus aureus*, on which the cloned vector or the use of strain 146 can be a powerful biological tool for the breakdown of casein.
Up to our knowledge, this is the first study that discusses casein breakdown products by *L. lactis* and their activity against staphylococci.

Acknowledgement:

Many thanks to Dr. Torsten Kleffmann (Centre for Protein Research, Biochemistry, The University of Otago) for helping in mass-spectrometry.

Reference:

du Toit, E. A. & Rautenbach, M. (2000). A sensitive standardised micro-gel well diffusion assay for the determination of antimicrobial activity. *Journal of Microbiological Methods* **42**, 159-165.

Hugenholtz, J., Van Sinderen, D., Kok, J. & Konings, W. N. (1987). Cell Wall-Associated Proteases of Streptococcus cremoris Wg2. *Appl Environ Microbiol* **53**, 853-859.

Laan, H. & Konings, W. N. (1989). Mechanism of Proteinase Release from Lactococcus lactis subsp. cremoris Wg2. *Appl Environ Microbiol* **55**, 3101-3106.

Monnet, V., Le Bars, D. & Gripon, J. C. (1987). Purification and characterization of a cell wall proteinase from Streptococcus lactis NCDO 763. *J Dairy Res* **54**, 247-255.

Reid, J. R., Moore, C. H., Midwinter, G. G. & Pritchard, G. G. (1991). Action of a cell wall proteinase from Lactococcus lactis subsp. cremoris SK11 on bovine alpha s1-casein. *Appl Microbiol Biotechnol* **35**, 222-227.

Smid, E. J., Driessen, A. J. & Konings, W. N. (1989). Mechanism and energetics of dipeptide transport in membrane vesicles of Lactococcus lactis. *J Bacteriol* **171**, 292-298.

Tan, P. S. T., Chapot-Chartier, M.-P., Pos, K. M., Rousseau, M., Boquien, C.-Y., Gripon, J.-C. & Konings, W. N. (1992). Localization of Peptidases in Lactococci. *Appl Environ Microbiol* **58**, 285-290.