Project number: 5842
Funding source: Enterprise Ireland
Date: March 2014
Project dates: Jan 08 – Sept 2011

Safe-formula

Key external stakeholders:

Infant formula manufacturers

Practical implications for stakeholders:

- Milk proteins can be digested by commercial enzymes to release antimicrobial peptides capable of inhibiting a range of pathogens. This is of significance to infant milk formula manufacturers where *Coronobacter sakazakii* is a contaminant of powdered infant formula milk. This pathogen has been associated with necrotizing enterocolitis, bacteraemia and a rare form of infant meningitis

Main results:

- An enzymatic method has been developed to release the antimicrobial peptide Caseicin A (IKHQGLPQE) from casein, based on hydrolysis with the commercial enzyme Thermoase PC10F.
- A second antimicrobial peptide (VFGKEKVNE) was identified in the casein hydrolysate.
- Food trials with Thermoase PC10F hydrolysates demonstrated a significant reduction in pathogen growth compared to controls.

Opportunity / Benefit:

Antimicrobial peptides for inclusion in infant milk formula to prevent the growth of *Coronobacter sakazakii*.

Collaborating Institutions:

Teagasc, University College Cork

Teagasc project team: Prof. Paul Ross (PI), Catherine Stanton, Caitriona Guinane, Robert Kent
External collaborators: Prof. Ger Fitzgerald, Colin Hill, Sarah Norberg, University College Cork,

Contact Catherine Stanton Email: catherine.stanton@teagasc.ie.

1. Project background:

Cronobacter (formerly *Enterobacter*) *sakazakii* is an emerging pathogen that has received attention as a causative agent of infant meningitis and septicemia. This organism has proved problematic for dairy ingredient manufacturers since dried infant formula has been implicated as a source of transmission (resulting in product loss and even recall). We have identified casein-derived peptides with activity against the pathogens *Cronobacter sakazakii*, *Staphylococcus aureus* and Salmonella. This project investigated antimicrobial peptides released from casein peptides following enzyme cleavage. Antimicrobial fractions were generated from a bovine casein hydrolysis, using the commercial enzyme Thermoase PC10F. The hydrolysis process was conducted at both lab-scale and pilot scale and the antimicrobial activity of the resulting powders were assessed for their efficacy in prevent pathogen growth in a number of food trials.

2. Questions addressed by the project:

- Can we develop an enzyme system for the release of the antimicrobial peptide Caseicin from bovine casein?
- Can we identify any further antimicrobial activity in such a system?
- Can we demonstrate the efficacy of the antimicrobial peptide in a food system?

3. The experimental studies:

Thermoase PC10F (Amano Enzymes) was added to the casein substrates at varying concentrations (from 0.1-2.0% w/v) and incubated at 37-65°C for 6-20h, in order to optimise hydrolysis conditions. The hydrolysates were heated to 95°C for 20 minutes to inactivate the enzyme prior to filtering through a size-exclusion (3kDa) spiral cartridge filter. The optimal conditions for release of the antimicrobial Caseisin A was determined to be 20h hydrolysis at 37°C with a final enzyme concentration of 5% w/v.

The resulting filtrates were analysed by MALDI-TOF Mass spectrometry (MS) and Reverse phase-HPLC (RP-HPLC) to determine the presence of the caseicin A peptide. Lab-scale hydrolysates were freeze-dried and pilot-scale hydrolysates (10L volumes) were spray-dried, to produce a bioactive powders for assessment in food trails.

A series of food trials including skim milk powder and powdered infant formula were undertaken, where the powders were spiked with pathogens and to this a level of 10% w/v <3kDa freeze-dried Thermoase PC10F powders was added. One strain of Cronobacter, 3 strains of *Staphylococcus aureus* and 1 strain of *Salmonella typhimurium* were investigated.

4. Main results:

- The antimicrobial peptide caseicin A was successfully liberated from casein using an industrial-grade and commercially available enzyme Thermoase PC10F.
- Freeze-dried filtrates of the thermoase hydrolysates were found to reduce the pathogens by 10^{1}-10^{3} CFU/mL over a 6h period, in infant formula when added at 10% w/v.

5. Opportunity/Benefit:

These results indicate that milk proteins can be a valuable source of antimicrobial proteins, with potential application for the control of pathogenic microorganisms in infant formula, and indeed other food systems.

6. Dissemination:

Main publications:

- Guinane, C Guinane CM, Kent RM, Norberg S, Hill C, Fitzgerald GF, Stanton C, Ross RP. (2011) 'Host Specific Diversity in Lactobacillus johnsonii as Evidenced by a Major Chromosomal Inversion and Phage Resistance Mechanisms' *PLoS One*. 6(4):e18740
- Kent RM, Guinane CM, O'Connor PM, Fitzgerald GF, Hill C, Stanton C, Ross RP. (2012) 'Production of the antimicrobial peptides Caseicin A and B by Bacillus isolates growing on sodium caseinate'. *Lett Appl Microbiol*. 55(2):141-8.
- Norberg, S., O'Connor, P.M., Stanton, C., Ross, R.P., Hill, C., Fitzgerald, G.F. and Cotter P.D (2011). 'Altering the composition of caseicin A and B as a means of determining the contribution of specific residues to antimicrobial activity'. *Applied and Environmental Microbiology* 77(7):2496-501.
- Norberg S, Stanton C, Ross RP, Hill C, Fitzgerald GF, Cotter PD. (2012) 'Cronobacter spp. in powdered infant formula'. *J Food Prot*. 75(3):607-20.

7. Compiled by: Sheila Morgan

Contact Catherine Stanton Email: catherine.stanton@teagasc.ie.