INCO COPERNICUS News Issue 3

Quality Control in Dairy Industry January 2001

Editorial

Thank you for reading the latest newsletter from Copernicus project PL 979012, aimed at up-dating procedures for detecting microorganisms in milk and milk-based products and developing new antibody-based tests for Listeria in milk products. The project consortium, consisting of academic researchers and the food industry, has been extremely active in research and in exchange of information, materials and personnel. Presentations at international scientific meetings have been made, including contributions to the Vth Agri-Food Antibodies meeting in Norwich, UK. Developments in biosensor technology (well represented in the current project) are fulfilling expectations as shown by real-time detection of analytes at high sensitivity. Two points are becoming clear. (i) the use of antibody-based methods in food analysis is increasing, and that (ii) the rate-limiting step in further exploitation is the development of new antibodies.

Novel antibodies of new specificities and affinities, antibodies able to tolerate extremes of solvents and pH, and antibodies more suitable for use in new formats are all needed, yet antibody production remains difficult, time-consuming and requiring considerable expertise even for standard applications. The weapons in the armoury of the immunochemist today would be recognisable to scientists from past decades. In spite of these problems, antibody production is regarded by some as routine! Research aimed at identification of novel antibodies (illustrated in the present project by using selected peptides as haptens) has exciting potential, but we need to learn much more about how to direct antibody specificity and about which factors determine antibody production against particular targets. We are beginning to realise the benefits of immunochemistry in food safety, in quality assurance of food, and in food research. Yet the power of the immune system has yet to be exploited to even a fraction of its full potential.

Professor Mike Morgan University of Leeds, UK.

A pathogenic life cycle of Listeria monocytogenes

L. monocytogenes is a facultative intracellular bacteria that invades, replicates and multiplies in a variety of mammalian cells including macrophages, epithelial cells and fibroblast cells. It has been observed that it has a very unusual life cycle with only a few species of bacteria like *Shigella flexneri* and *rickettsia* using the same method of intra- and intercellular spread through interaction with f-actin. A family of bacterial cell-wall surface proteins, called internalins (described below), are present at the cell surface of *L. monocytogenes* which promote its entry into epithelial cells and hepatocytes. *L. monocytogenes* also uses the bodys own defense systems to gain entry into the cytoplasm of infected cells. It can be phagocytosed, enclosing the bacterium in a subcellular organelle called a phagolysosome, which is a toxic and hostile environment for most bacteria. The pH inside the phagolysosome drops which in turn activates listeriolysin O (LLO) and the bacterial metalloprotease (Mpl) resulting in lysis of the organelle within 30 minutes, allowing the bacterium to escape into the cytoplasm.

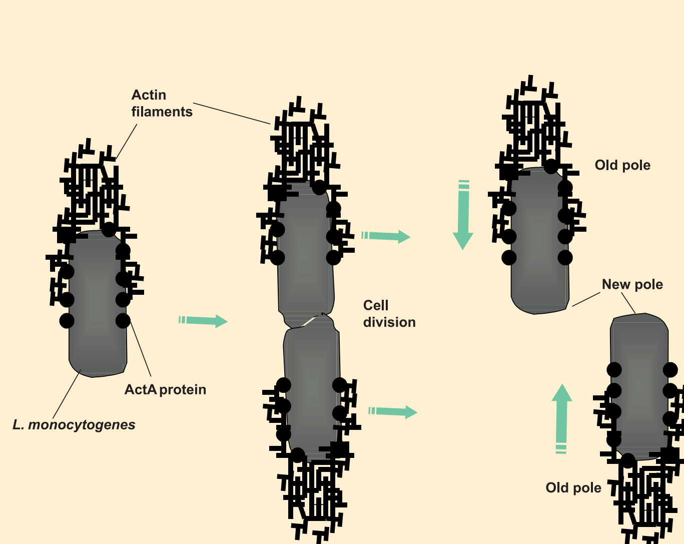

Figure 1. Cell cycle distribution of ActA protein (black dots) and actin filaments (black lines) in L. monocytogenes. ActA, required for the bacterial induced actin assembly, is distributed around the cell except at the pole following cell division. The direction of movement of the new cells is therefore in opposite directions as indicated by the arrows.

All pathogenic strains of Listeria produce listeriolysin O as entry of the bacteria into the cytoplasm of the host cell is required for pathogenesis.

Within the cytoplasm, *L. monocytogenes* can proliferate and becomes surrounded by a high density of cytoplasmic actin. Actin is the most abundant protein in the cytoplasm of mammalian cells, accounting for 10 to 20 % of the total cytoplasmic protein. It exists either as a globular monomer (G-actin) or a filament (F-actin), the latter formed by head to tail polymerisation of asymmetric monomers. As the cell divides or proliferates, ActA protein (see Figure 1) and actin filaments are distributed around the cell with the exclusion of the new pole. As the septation region is devoid of ActA, the movement of the progeny is usually in opposite directions to each other.

Using this actin-based motility process, the bacterium migrates to the peripherary of the cell where it creates a protrusion or "filopod" into the adjacent cell. This filopod can be ingested into the adjacent cell forming a now double membraned phagolysosome (see Figure 2) where the life cycle begins over again.

Bacterial Entry into the host cell

Entry into the host cell involves the bacterial surface proteins including InlA (internalin) and InlB. The receptor for InlA is the cell adhesion molecule E-cadherin. InlB-mediated entry requires activation of the host cell protein phosphoinositide (PI) 3-kinase, probably in response of engagement of a receptor.

Internalin A (InlA)

InlA is an 80 kDa protein needed for entry into the human intestinal epithelial cell line Caco–2. Expression of InlA in the non-invasive bacterium *L. innocua* renders the organism capable of invading Caco–2 cells suggesting that InlA may be sufficient for entry. The primary structure of internalin can be characterised by two different regions of repeats. The first region is made of fifteen 22 amino acid leucine-rich repeats (LRRs). The second region is formed of three consecutive repeats, two of 70 amino acids, one of 49 amino acids. It also contains a signal sequence and a carboxy-terminal hydrophobic region proceeded by the pentapeptide LPTTG.

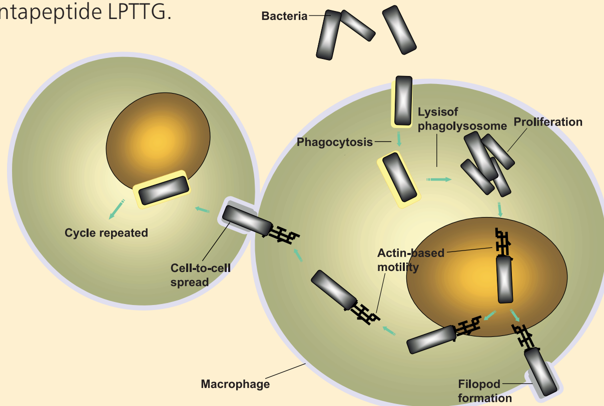

Figure 2. Life cycle of L. monocytogenes in host cells.

It has been suggested that internalin is anchored to the cell wall peptidoglycan of epithelial tissues by covalent linkages via the threonine residue of the conserved motif LPXTG (where X is any amino acid). It was demonstrated that the cellular receptor for internalin is E-cadherin, a Ca2+-dependent cell-cell adhesion molecule that plays a critical role in cell sorting during development, formation of intercellular junctions, polarization of epithelial cell layers, and maintenance of adult tissue architecture. By using anti-E-cadherin antibodies in a microtiter plate binding assay it was examined whether binding of Caco–2 cells to internalin was strictly E-cadherin-dependent. Pretreatment of a Caco–2 cells suspension for 50 minutes at 37 °C with 10 μg/ml or 100 μg/ml of anti E-cadherin antibodies (DECMA–1) resulted in 50 % and 96 % reduction, of Caco–2 cell binding, respectively. Hence it was concluded that the ability of Caco–2 cells to bind to internalin is mostly E-cadherin mediated.

Internalin B (InlB)

InlB, a 630 amino acid protein encoded by a gene found downstream of InlA, has been shown to mediate entry into cultured hepatocytes and some epithelial and fibroblast cell lines, including HeLa, HEP–2, CHO, L2, and Vero. InlB and the products of at least six other genes in *L. monocytogenes* have leucine rich repeats (LRRs) similar to those in InlA, forming the "Internalin family of proteins". The mechanism of association of InlB with the bacterial surface is different from that of InlA. InlB protein does not contain any known cell-surface anchoring region, such a hydrophobic C-terminal region or an LPXTG motif. Instead InlB is attached to the bacterial surface via a 232 amino acid repeat region beginning with the sequence glycine-tryptophan (GW) that is also present in a newly identified surface-associated bacteriolysin of *L. monocytogenes*, called Ami. Addition of GW repeats to the C-terminal of InlB improves anchoring of the protein to the cell surface. By constructing isogenic chromosomal deletion mutants in the InlAB locus, it was assessed the rate of InlB in invasiveness of *L. monocytogenes*. It was found that InlB is required for entry of *L. monocytogenes* into hepatocytes, but not into intestinal epithelial cells. It was also noted that even though InlB is not required for entry into intestinal epithelial cells, the mutant InlB used was two fold less invasive than the wild-type strain serotype 1/2a (EGD). InlB-mediated entry into certain hepatocytes, Vero cells, some other cells probably occur through a receptor distinct from E-cadherin, however this receptor is currently under investigation.

Listeriosin O (LLO) and metalloprotease (Mpl)

When inside the cell, the cell recognizes the endocytosed vacuole as a target for degradation. How this recognition occurs is not fully understood, but the sudden drop in pH activates LLO and Mpl; the latter cleaving the bacterial-secreted phospholipase C (proPC–PLC) to its active form with vacuolar lysis occuring rapidly.

Intracellular spread of Listeria: An actin-based process

Once escaped from the phagolysosome, the bacterium resides within the host cytoplasm where it harnesses the host machinery required for actin assembly, generating a comet-like tail, which propels the bacterium within the host cell. It is this actin-based motility that enables listeria to spread infection from one cell to the next via a membrane-bound protrusion.

ActA protein

Act A, a 639 amino acid protein with a C-terminal hydrophobic region involved in anchoring to the membrane, is needed for polymerization of host actin and movement. Genetic studies have shown that ActA possesses two regions important for motility. The NH_2-terminal region (1—262) appears solely responsible for actin nucleation, a low-probability process in which three actin monomers combine simultaneously to form a thermodynamically unstable trimer, the first step in the formation of filaments. The central domain (263—390), which contains four proline rich repeats, controls the rate of movement. The proline-rich repeats of ActA form the binding site for vasodilator-stimulated phosphoprotein (VASP) one of the many cellular proteins including alpha-actinin, profilin, cofilin, vinculin and the Arp2/Arp3 complex, which is involved in actin-based movement.

VASP (Vasodilator-stimulated phosphoprotein)

It is associated with microfilaments in focal contacts and lamellae in spreading cells. This tetramer of identical 40-kDa subunits binds to profilin, a host cell component which binds to actin monomers in a one--to-one complex, alters the confirmation of the actin monomer, and accelerates the exchange of ATP for actin-bound ADP and ActA. ActA has four potential VASP-binding sites and VASP has 16 potential profilin-binding sites. As a result, this two-step binding-mechanism allows one ActA molecule to concentrate up to 64 profilin molecules, strongly amplifying the ability of listeria to promote the assembly of actin filaments.

Arp2/3 complex

ActA protein also contains a positively charged amino acid sequence thought to be involved in promoting actin nucleation by attracting negatively charged actin monomers. The multiprotein complex isolated from platelet extracts, containing the actin-related proteins Arp2 and Arp3, is sufficient to initiate actin polymerization at the surface of listeria, most likely by interacting with ActA. The Arp2/3 complex has been observed to remain bound to the pointed ends of nucleated filaments and is uniformly distributed throughout the actin tail while the bacterium is moving. Although the direct binding of Arp2/3 to ActA has not been demonstrated, the domain of ActA that was found to synergize with Arp2/3 in nucleating actin assembly *in vitro*, maps to the N-terminus of the protein, the same region of ActA that has been shown to be essential for nucleation of actin assembly on the surface of the bacterium within the cytoplasm of the mammalian cell. Indeed, when ActA and Arp2/3 are mixed together with monomeric actin, the nucleation phase of actin assembly, which is normally slow, is essentially instantaneous. It is thought that Arp2/3-dependent capping of actin filaments may also enhance their stability by slowing disassembly from the pointed end of the polymers.

Alpha-actinin and cofilin (ADF)

Other proteins such as capping proteins, ADF/cofilin and alpha-actinin play an important role in enhancing filament turnover, i.e. in increasing the site-directed barbed end-growth. Alpha-actinin, a homodimer with an actin-binding site on each monomer, has the ability to cross-link actin filaments. It has been shown that microinjection of infected cells with a 53-kDa proteolytic fragment of this protein, causes the disappearance of bacteria-containing filopodia and an arrest in movement, indicating that this fragment acts as a dominant negative protein and that cross-linking of actin filaments is important for growth. Cofilin(ADF), a small G- and F-actin binding protein, increases the rate of actin depolymerization and shortens actin tails in human platelet extracts, while addition to highly diluted platelet extracts increased the rate of bacterial movement. From these results it was suggested that cofilin may affect bacterial motility by stimulating the rate of actin filament turnover.

Paul Leonard

Basic principle of biosensors for bacteria detection and their use in *Listeria monocytogenes* assay

In our previous issue we informed you about *Listeria* outbreaks in Europe and the USA. Listeriosis still represents a serious risk for people and the real danger is underlined by infection in France this year. Listeriosis outbreaks have been associated especially with dairy products, coleslaw, hot dogs and deli meat (see INCO COPERNICUS News, Issue 2, Listeria in USA — Occurrence, Control, Analysis).

Because of the low recovery of the bacterium by direct plating, *L. monocytogenes* is most often recovered by a two-step enrichment/isolation method (see INCO COPERNICUS News, Issue 2, Isolation of Listeria from foods). The advantage of this technique is high sensitivity and low cost, but at least several days for total detection are required.

Several methods to shorten the time need for total analysis were done including nucleic-acid based and antibody based methods. These methods still required at least 24 h for total analysis (see INCO COPERNICUS News, Issue 2, Rapid methods for detection of Listeria in foods).

Biosensors for bacteria detection

Biosensor analysis seems to be a very useful tool to overcome the main disadvantage of these methods — the long time needed. Biosensors for bacterial detection generally involve biological recognition components such as receptors, nucleic acids and antibodies in intimate contact with an appropriate transducer. For biosensors construction, change of the mass, optical, electrochemical or thermal quantity can be measured. In addition, biosensors could be further classified with direct or indirect format of detection. For direct detection, measurement of biospecific complex formation in real time is characteristic. In indirect detection, a preliminary biochemical reaction takes place and only the product of the reaction is measured.

Principles of detection

In amperometric biosensors, the antibody is labelled with an enzyme. The most frequently used enzymes are especially peroxidase and alkaline phosphatase. Using alkaline phosphatase, p-aminophenyl phosphate is used as a substrate of the enzyme. Alkaline phosphatase hydrolyses p-aminophenyl phosphate to p-aminophenol, which is then monitored amperometrically on the electrode. Current flowing through electrode is then proportional to the amount of enzyme labelled antibody.

Alkaline phosphatase could be used also for ***optical detection***, when p-nitrophenyl phosphate could be used instead of p-aminophenyl phosphate. This compound is hydrolysed by alkaline phosphate and the colour change is measured.

Piezoelectric principle of detection is based on the change of resonance frequency of piezoelectric crystal due to a change of the mass on the crystal surface. The mass change is caused by interaction of antigen with antibody immobilised on the crystal surface.

Microbial metabolism usually results in a decrease of impedance, because of the presence of electrically conducting compounds. Thus, ***impedance decrease*** could be monitored using a bridge circuit. It is necessary to use a reference module, because of non-specific changes in the test module as a result of temperature changes, evaporation, changes in amount of dissolved gases and degradation of culture medium. The quantity conductance is in indirect relation the term impedance.

Evolvement or consumption of heat is characteristic for biological objects. In this case also the reference module is needed because of non-specific evolvement or consumption of heat.

Bioluminescent principle of detection is based on the fact that all bacteria contain ATP. The reaction between ATP and luciferase is very specific, sensitive and rapid; however interference form non-microbial ATP can be a problem.

Mass changes caused by binding of antigen to the antibody immobilised on the surface of SPR (Surface Plasmon Resonance) sensor chip result in a change of refractive index of light (BIACORE 2000 and 3000, see Fig. 1 and 2). This output value against time reveals the rate at which the antigen-antibody complex forms (see INCO COPERNICUS News, Issue 2, Characterisation and Application of Immunoassay Reagents for Rapid Detection of Listeria monocytogenes using Biosensor Technology). The advantage of this system is the possibility to monitor antigen-antibody kinetics.

Many of these principles of detection were used in commercial biosensors (see Table 1)

Fig. 1: BIACORE 2000

Table 1: Commercial instruments for bacteria detection

Instrument	Detection principle	Detection limit (cells.ml–1)	Analysis time
Midas Pro (Biosensori SpA, Italy)	Amperometric	10^6	20 min
The PZ 106 Immuno-biosensor System (Universal Sensors, USA)	Piezoelectric	10^6	40 min
Hybritech, USA	DNA probe	—	—
Bactometer (Bactomatic, USA)	Impedimetric	10^5	3—8 h
Malthus 2000 (Malthus Instrumentns, UK)	Conductance	10^5	8—24 h
Unilite (Biotrace, UK)	Bioluminescence	10^3	15 min
Lumac Biocounter (Lumac B.V., Netherlands)	Bioluminescence	10^3	20 min
Coulter counter (Coulter electronics, Canada)	Coulter counter	5×10^4	30 min
Thermal activity monitor (Thermometric, UK)	Microcalorimetric	10^5	3 h
BIA-core (Pharmacia, Sweden)	Surface plasmon resonance	10^5	1 h
Vitek Automicrobic System (BioMerieux Vitek, USA)	Optical	10^4	4 h

Fig. 2: BIACORE 3000

Biosensors for *Listeria monocytogenes*

Recently several biosensors for *Listeria* detection were developed. Biosensors for *Listeria* detection could be divided into three main groups — optical, piezoelectric and electrochemical.

For ***optical detection*** a 200-mer fragment of the *fla*A gene from *L.* monocytogenes with amplification using PCR (polymerase chain reaction) was developed.

If A511 bacteriophage containing the genes encoding luciferase infects *L. monocytogenes*, this bioluminescent phenotype can be conferred on previously non-bioluminescent bacteria. Using this bacteriophage it was possible to detect one viable cell of *L. monocytogenes*/gram within 24 h.

A ***piezoelectric crystal*** was also used for *L. monocytogenes* detection. Using this method of detection a displacement assay was used. This means that the antigen *Listeria* was immobilised on the crystal surface. The addition of antibody resulted in immunocomplex formation with increase in mass and decrease in resonance frequency. Then *Listeria* cells were added to the system causing displacement of antibody from the immunocomplex, resulting in increase of frequency. There is a little reaction with non-specific antigen — *Vibrio*. The calibration was linear in the range 2.5 × 105 to 2.5 × 107 cells/crystal and the time need for analysis was less than 15 min.

The ability of haemolytic organisms to rupture the lipid bilayer of liposomes was applied with ***amperometric detection***. Liposomes contain a mediator, which is released after rupture and then is detected on the electrode at fixed potential. Non-haemolytic strains such as *E. coli* or *L. welshimeri* were not able to disrupt the liposome and no signal was observed.

Recently, evaluation between an ELISA method and amperometric detection of *L.* monocytogenes was done. Both methods are based on biospecific antibody-antigen interaction. The overall assay time for amperometric detection, including antibody immobilisation and blocking steps, was 3.5—4 h, which is significantly faster then other techniques. This techniques offers only steady-state signal after equilibrium is established; kinetic data cannot be obtained.

Publications

Greifová, M., Melišová, D., Horáková, K.: Occurrence and surviving of *Listeria monocytogenes* in milk and milk products. Dairiing, 31(2), 2000, p. 40—43 (in Slovak language).

Horáková, K.: Report from INCO COPERNICUS meeting in Žilina. Dairiing, 31(2), 2000, p. 36 (in Slovak language).

Karasová L., Fukal L., Rauch P., Wyatt G., Brett G., Morgan M.R.A.: Perspectives of *Listeria monocytogenes* immunoassay detection, Czech J. Food Sci. 18 (2000) 268—269. ISSN 1212–1800.

Tomáška, M., Slottová, A., Kontová, M., Gondová, B.: *Listeria* in cheese — occurrence and monitoring, in: *Proceedings of lectures and posters from Syrotech 2000, 3rd—5th May 2000 Žilina* (ed.: K. Herian), 170—177, Dairy Research Institute, Žilina, 2000.

Table 2: ELISA method for Listeria monocytogenes detection

System	Time (h)	Working range (cells.ml^{-1})	Detection limit (cells.ml^{-1})
DA (antigen)	6	3×10^7—1×10^6	1×10^5
DS (goat)	7	2×10^7—3×10^5	4×10^4
DS (rabbit)	7	2×10^7—4×10^5	3×10^3
DS (mouse)	7	2×10^7—8×10^5	8×10^4
Biotin-avidin	8	2×10^7—3×10^4	3×10^3
IS (goat)	8	2×10^7—8×10^4	9×10^3
IS (rabbit)	8	2×10^7—9×10^3	3×10^3

DA=Direct assay, DS=Direct sandwich, IS=Indirect sandwich

ELISA assays

Several analysis methods were done using alkaline phosphatase (AP)-labelled antibodies including: monoclonal (mouse anti-*L. monocytogenes*) and polyclonal (goat and rabbit anti-*L. monocytogenes*) antibodies. Colour changes by the presence of alkaline phosphatase were measured at 405 nm. Direct assay and direct sandwich assays were of low sensitivity, so experiments were carried out to amplify the response using biotin-avidin complex and by indirect sandwich assay. Results of these experiments are summarised in Table 2.

Table 3: Amperometric assays for Listeria monocytogenes detection

System	Time (h)	Working range (cells.ml^{-1})	Detection limit (cells.ml^{-1})
DA (antigen)	3	unreliable	unreliable
DS (goat)	3.5	1×10^6—1×10^4	8×10^3
DS (rabbit)	3.5	1×10^6—1×10^3	9×10^2
DS (mouse)	3.5	1×10^6—1×10^5	1×10^3
Biotin-avidin	4	unreliable	unreliable
IS (goat)	4	1×10^6—7×10^3	1×10^3
IS (rabbit)	4	1×10^6—1×10^3	9×10^2

DA=Direct assay, DS=Direct sandwich, IS=Indirect sandwich

Amperometric assays

For further improvement amperometric detection using screen-printing electrodes was used. Antibodies were immobilised by covalent coupling with carbodiimide. The effect of non-specific binding was done using cells of *Bacillus cereus* and a value less then 1% was found in all cases. Results of these measurements are summarised in Table 3.

As can be seen from these two tables, amperometric detection leads into assays with lower detection limit, and shorter times were achieved by immobilising *L. monocytogenes* cells followed by incubation with goat anti-*L. monocytogenes*-AP.

Direct sandwich assays were done by immobilisation of goat and rabbit anti-*L. monocytogenes* followed by incubation with *L. monocytogenes* cell. From these two tables, it is clear that amperometric biosensor is more sensitive in comparison with ELISA method.

Ján Tkáč

NEXT MEETINGS will be held

In Zvíkov June 2001
In Prague October 2001

Recently changed

Professor Mike Morgan left Institute of Food Research in Norwich for Leeds University. New e-mail address:
M.Morgan@food.leeds.ac.uk
e-mail address of partner P6 has changed from ***vumza@netlab.sk*** to ***vumza@vumza.sk.***

Minutes of INCO COPERNICUS project meeting in Norwich 12th—13th September 99

Present

ICT, Prague	Pavel Rauch, Ludmila Karasová
IFR, Norwich	Mike Morgan, Gary Wyatt, Gary Brett
STU, Bratislava	Katarína Horáková, Andrea Šovčíková, Zuzana Laurincová
DCU, Dublin	Richard OKennedy, John Quinn
Milcom, Prague	Peter Roubal
DRI, Zilina	Martin Tomáška

Introduction

1. Participants were welcomed by Pavel Rauch. Gary Wyatt outlined the programme and welcomed partners to Norwich.

2. Pavel reported changes in Brussels with Renney replacing Aspilla as project officer. Partners were reminded to send individual reports in Word 6.0 or 7.0 and by post in December. Reports should contain (i) summary report of 2—4 pages, (ii) detailed scientific results suitable for dissemination between partners and (iii) annexes — publications, meetings, etc.

3. Katarina reported that she did not receive the last minutes.

Scientific Presentations

4. Institute of Chemical Technology, Prague, Czech Republic

Pavel Rauch reported that so far antisera from 8 MAPs has been tested as well as different microtitration plates and coating conditions. The immunisation scheme was as before. In the first experiment plates were coated with *L. monocytogenes* and *L. innocua* by sedimentation from 50 % ethanol. Anti-MAP 9 gave the best results although there were some problems with the coating.

Coating plates with MAP revealed differences in the effect of incubation time and temperature. Long term storage of the MAPs at –20 °C also had a detrimental effect on antibody binding, preservation reagents had no effect. Assay performance was also affected by the use of BSA in the buffer and the type of microtitration plate. Fresh reagents are important.

There was some discussion on the relative merits of monoclonal and polyclonal antibodies within the aims of the project and the possibility of steric factors on the recognition of MAPs by antibodies.

5. Institute of Food Research, Norwich, UK

Gary Wyatt outlined the tasks of IFR; producing a new batch of MAPs, producing derivatised peptides, immunisation of mice for Mab production and producing articles for the newsletter. Dot-blots with the MAP immobilised onto nitrocellulose and probing with anti-MAP antisera showed good recognition of the respective peptides by antisera to MAPs 2, 4, 6 and 9. All antisera seemed to bind to MAP 3. There was no binding to MAPs 1, 7, 8 and 10. These results correlated well to those obtained by ELISA at ICT. At IFR a single rabbit has been immunised with a mixture of Inl MAPs and another with a similar mixture of ActA MAPs. Two groups of mice have been immunised in the same way. Bleeds from all animals show encouraging patterns of binding to their respective peptides. There was some discussion about how well the immune response correlates to the parameters governing the choice of peptide sequence.

6. Slovak University of Technology, Bratislava, Slovakia

Katarína Horákova reported on progress on the production of a new pathogen handling laboratory. 22 strains of *Listeria* had been received from GW and their growth characteristics on different media have been evaluated, not all selective media are compatible with ELISA. The media tested were (i) brain heart infusion (Merck), (ii) *Listeria* enrichment broth (Merck), (iii) UVM1 formulation of *Listeria* enrichment broth (Merck) and (iv) Fraser broth without ferric ammonium citrate. All cultures grew in all media although GW reported that some strains of Listeria grew poorly on Oxford agar. There was further discussion about the temperature needed to inactivate *Listeria* and methods of long term storage.

7. Dublin City University, Dublin, Republic of Ireland

John Quinn reported that their involvement so far had been restricted by the lack of suitable antibodies. Passive absorption is insufficient to attach protein to the crystal surface. Pure protein is needed and the immobilisation is by amine coupling, using charge attraction to pre-concentrate the protein onto the crystal surface. The pH of the solution should be below the isoelectric point of the protein, for InlB pH 4.1 was used. For antisera screening, with immobilised InlB a very small response was obtained, on the limit of sensitivity. Future assays will look at direct detection of pasteurised *Listeria* cells, inhibition assays for indirect cell detection, affinity/kinetic characterisation of Mabs, and repetition of assays in crude enrichment broth.

There was further discussion about assay format, the problem of binding large cells and the necessity of high avidity antibodies.

JQ outlined progress in the development of fibre optic based probes.

8. Milcom, Prague, Czech republic

Peter Roubal outlined the current state of the quality of dairy products in the Czech Republic. The total bacteria count had dropped from 1997 to 1998. Numbers of somatic cells and levels of antibiotic residues had remained constant. Although dropping significantly between 1997 and 1998 the levels of psychotrophic bacteria are still the main concern. By the end of the project it is hoped to be able to present similar quantitative data for *Listeria* levels. PRo outlined national limits and detection methods used presently.

9. Dairy Research Institute, Žilina, Slovakia

Martin Tomáška outlined the objectives of DRI; the selection of suitable cultivation methods, validation of these methods and the subsequent analysis of samples. The procedure of ISO 11290–1:1996 was outlined, which can take 10 days to produce a result with around 200 cultivations per sample. False positives are sometimes observed and some *L. monocytogenes* cultures do not grow well on some agars.

Confirmation tests were described; for *Listeria* these are microscopic examination, Gram staining, catalase reduction and motility test; and for *monocytogenes*, haemolysis, carbohydrate utilisation and CAMP test.

70 samples of milk products from suspect areas and 5 brine bath samples were analysed. 32 % of all milk products were found to contain *Listeria*. *L. monocytogenes* was found in 5 % of analysed samples, but only in hard, semi-hard and steamed cheese.

Plans for the future include, improvement of analytical skills for the detection of *L. monocytogenes*, to continue sample analysis, compare results with other laboratories and to select critical control points during processing to analyse for *L. monocytogenes*.

Other matters

10. Future work

GW outlined the project objectives for all partners. All tasks are on schedule. Lida Karasova will be visiting IFR for 2 months to produce Mabs and to begin antibody characterisation. DCU will begin to characterise reagents, using derivatised peptides, MAPs and whole proteins. They will also need enough material for ELISAs to compare with Biacore results. The issue of steric hindrance will be investigated. STU will prepare *Listeria* cultures ready for the meeting in Dublin and will begin using ELISAs as they become available.

11. Report

Contributions should be sent to Prague as soon as possible. It was agreed that only short summary reports are needed from Milcom and DRI.

12. Newsletter

It was decided that all partners will send items to KH and she will decide which to include in which issue. So far articles had been received form JQ and GW. In view of the problems experienced in delivery of some articles it was agreed that all contributions must go to KH. The minutes of each meeting will also be sent to KH. There have been problems in receiving payment for the newsletter from each partner. GW to see IFR accounts dept.

Proposed future articles are:*Listeria* in Czech/Slovak Republics — PR (maybe PRo better)/MT; *Listeria in UK — GW; Listeria in Ireland — ROK*

Participants of Inco Copernicus meeting organised in Norwich in the lecture hall (from left to right Mike Morgan, Petr Roubal, Gary Brett, John Quinn, Richard O'Kennedy, Andrea Šovčíková, Gary Wyatt, Katarína Horáková, Pavel Rauch, Zuzana Laurincová and Lida Karasova). Pictured by M. Tomáška.

Minutes of INCO COPERNICUS project meeting in Dublin 25th—26th May, 2000

Chairman: Pavel Rauch (**PRa**, ICT, Prague)
Present: Richard O'Kennedy (**ROK,** DCU, Dublin), John Quinn (**JQ,** DCU, Dublin), Paul Leonard (**PL,** DCU, Dublin), Stephen Hearty (**SH**, DCU, Dublin); Lida Karasová (**LK**, ICT, Prague), Barbora Micková (**BM** , ICT, Prague); Katarína Horáková (**KH**, STU, Bratislava), Andrea Šovčíková (**AS**, STU, Bratislava); Martin Tomáška (**MT**, DRI, Zilina); Petr Roubal (**PRo**, Milcom, Prague); Mike Morgan (**MM**, Uni. Leeds); Gary Wyatt (**GW**, IFR, Norwich)

The participants were welcomed to Dublin by Richard OKennedy, head of School of Biotechnology at Dublin City University. In order to be sure that everyone was familiar with the project, ***GW*** gave an overview of the aims, objectives and scientific approach of the project. He also presented a diagram summarising the possible flow of work from the initial anti-MAP (multiple antigenic peptide) antibodies, through to final recognition of *Listeria* cells; likely decision points involved in the process were included as a basis for discussion at the meeting.

Scientific Presentations:

1. Institute of Chemical Technology, Prague, Czech Republic

PRa summarised the work carried out in Prague with their polyclonal antisera. There had been problems with some makes of microtitre plates — Costar 9018 were found to be best. MAPs numbers 2, 4, and 6 gave the best competition with their respective antisera, with I50 in the range 100—250 ng/ml. MAPs numbers 1, 5, 7, and 9 gave moderate to poor competition. Cross-reactivity of each anti-MAP serum with all of the MAPs had been calculated. Using anti-MAP 1 serum with MAP 1-coated plates, competition with whole Listeria cells had been achieved, but non-*Listeria* also appeared to compete in this system. A similar result was found using MAP9 and its antiserum, and with whole cell extract as a plate coating. The effect of various blocking agents (BSA and gelatin) and Tween, had been tried.

LK described her training in monoclonal antibody production at IFR, Norwich, and results for initial characterisation of her anti-MAP hybridoma cell lines; work on these was continuing in Norwich.

2. Institute of Food Research, Norwich, UK

GW had successfully purified more internalin B. He showed dot-blots of the binding of polyclonal serum produced in Norwich to immunogens comprised of mixed MAPs, purified internalin B, and whole cell extract. Sera were tested in an ELISA against immobilised whole cells — Prague sera against MAPs 5 & 10 gave moderate binding to *L. monocytogenes*, a lesser binding against *L. innocua*, and low binding against an *E. coli* control; however, the sera produced in Norwich against mixed MAPs showed no binding to whole cells (the reason for this result was not clear). Norwich sera against whole cell extract and internalin B bound very well and moderately well, respectively, against cells. Selected antisera had been labelled with HRP and "sandwich" ELISAs developed; so far, the best assay had been achieved with anti-whole cell protein as both capture and detector antibody.

3. Slovak University of Technology, Bratislava, Slovakia

KH described an extensive study of the growth characteristics (specific growth rate, lag time, generation type, total growth)of various *Listeria* species in 9 different enrichment media and juices, using a micotitre plate method. The best growth environment for *L. monocytgenes* was found to be GTK medium; the non-*monocytogenes* species had more exacting growth requirements than *L. monocytogenes*. Production of the p60 virulence protein in these media was assessed using a commercial ELISA This test did not detect the non-monocytogenes species, and all *L. monocytgenes* were positive. Except recommended Palcam and Fraser, also LEB, BMI, UVM and NB No2 broths can be respectively used as they created optimal conditions for growth of 9 tested strains of *Listeria monocytogenes* and production of extracellular protein p60.

4. Dublin City University, Dublin, Republic of Ireland

PL described peptide-avidin conjugates made using the monomeric peptides (numbers 4 & 9) having a terminal cysteine made in Norwich. Results of the binding of the four Norwich polyclonal sera (see above) against these conjugates, and against MAPs, in ELISA and BiaCore formats were presented — the anti-Inl MAPs serum (R606) was particularly good; the others either did not bind or showed non-specific binding. Serum

R606 also bound to internalin B whole protein in the BiaCore system, as did the sera raised against whole cell extract and internalin B. The binding of a new Dublin antiserum against a peptide-avidin conjugate was found to be salt-dependent. However, a new Dublin serum raised whole internalin B gave excellent binding to whole *Listeria* cells — this result was therefore similar to that found in Norwich with their serum (see above).

5. Milcom, Prague, Czech republic

PRo described the Czech standard 560093 for *Listeria* isolation. This includes a recommended medium (Frazer broth) and various biochemical tests. He had also tried a commercially-available dipstick ELISA (Tecra "Unique") and found three positive raw milk samples using this test. Further comparison of the classical method with new methods would be carried out in conjunction with a reference laboratory. He then described Czech raw milk quality for the period 1997—1999 — total bacterial count had declined, but somatic cells and coliforms had risen.

6. Dairy Research Institute, Žilina, Slovakia

MT presented results of analysis for *L. monocytogenes* in Slovakia for 1999. 5.4 % of hard cheeses made from pasteurised milk were positive. It was not known if this was pasteurisation failure or re-contamination. *L. monocytogenes* was found in 7.6 % of raw cow's milk samples. He described the "RAPID L. MONO" L. monocytogenes test based on phospholipase activity on an agar containing a chromogenic substrate.

7. Institute of Food Research, Norwich, UK

GW described various dipstick formats that might be used for the assays developed in the project. These included the plastic "paddle" type, and lateral-flow devices. He also described possible antibody labels.

Evaluation of Progress

General discussion on progress followed, based around the flow chart mentioned above. It seemed that antibodies to peptides (MAPs and single peptides), whole virulence proteins (internalin B) and whole cell extracts had been successfully produced; some anti-MAP antibodies bound to the whole protein from which the sequences were derived, and some bound to cells, but a continuous connection the whole way through the flow as described in the chart had not yet been established. Some discussion centred on peptide binding epitopes, and the possibility of mapping this, followed.

Future Work

GW will transfer his working sandwich ELISA to Bratislava.
KH will transfer *L. monocytogenes* strains to Dublin.

Next meeting:

Bratislava, 23rd & 24th November, 2000

Other business

1. Newsletter — issue 3 would be in September/October. ***MM*** would provide the editorial, and articles would be written by ***GW***, ***MT***, ***PL, JT*** and ***LK/AS***.

2. Payments to partners — still not received from Norwich. ***GW*** would investigate.

3. Annual report — ***PRa*** asked for each partner to bring their report to the next meeting.

4. A tour of the DCU School of Biotechnology laboratories followed.

Participants (from left to right: Stephen Hearty, Paul Leonard, Gary Wyatt, John Quinn, Petr Roubal, Richard O'Kennedy, Lida Karasová, Katarína Horáková, Andrea Šovčíková, Mike Morgan and Pavel Rauch) of Inco Copernicus meeting organised in Dublin before Dublin City University…

… and in the meeting room during discussion. Pictured by M. Tomáška.

Posters:

Wyatt G.M., Karasová L., Brett G., Rauch P.: Specific detection of *Listeria monocytogenes* in foods: a novel approach using antibodies to peptides representing virulence proteins, Society for Industrial Microbiology meeting, Washington, April 16—19, 2000.

Karasová L., Fukal L., Rauch P., Wyatt G., Brett G., Morgan M.R.A.: Perspectives of *Listeria monocytogenes* immunoassay detection, Chemical reactions in foods IV, September 20—22, Prague 2000

Wyatt G., Aldus C., Peck M., Brett G., Karasová L., Rauch P.: Antibody-based detection of food-borne pathogens and toxins, Federal Food Safety and Nutrition Research Meeting, Otawa, Canada, 24—26 September 2000.

Wyatt G., Aldus C., Peck M., Brett G., Karasová L., Rauch P.: Antibody-based detection of food-borne pathogens and toxins, Agriculture and Agri-Food Canada Food Network 2000, Saint Hyacinthe, Canada, 28—29 September 2000.

Karasová L., Doležal J., Fukal L., Brett G., Rauch P., Wyatt G.M.: Characteristaion of antibodies to peptides representing virulence proteins of *Listeria monocytogenes*, XVII. Czech and Slovak Biochemical Congress, Praha, 7—10th September 2000.

LISTERIA MONOCYTOGENES AND LISTERIOSIS IN SLOVAKIA

This is a short review about *Listeria monocytogenes* and listeriosis monitoring and methodology used to lay stress on dairy industry in Slovakia.

Food safety system

The integrated approach between government and all segment and sectors of the food industry has been built in Slovakia to enhance food safety. Food industry has a responsibility to produce food that meets quality requirements. The maximum attention is paid to the hygienic quality. The principles of GHP, GMP, and HACCP were included in the national food legislation and application of these systems is obligatory for producers and distributors.

In 1998 the Microbiological part of the Food Codex of SR was issued. According to this document *L. monocytogenes* should be absent from 10 g samples of food determined for direct consumption having a_w higher than 0.92. However in some dairy and meat products this tolerance is even stricter, *L. monocytogenes* should be absent from 5 × 25 g of sample. If *L. monocytogenes* is detected then the product must be excluded from human consumption and withdrawn from the market. Therefore producers should regularly send their samples to the accredited laboratories for analysis of pathogens including *L. monocytogenes* to verify systems of quality. Further this evaluation, hygienic quality of food is inspected by governmental food control authorities.

Table 1: *Listeria monocytogenes* in slaughter animals, milk, food, and surfaces *(State Veterinary Administration of SR, 2000)*

Sample	1998		1999	
	Samples	% of Posit Samples	Samples	% of Posit. Samples
Cattle	50	0.0	33	15.2
Sow/Pig	22	13.6	56	8.9
Poultry	30	0.0	2	0.0
Another	5	0.0	6	0.0
Surface	40	10.0	1729	0.2
"Bryndza" (cheese)	212	0.0	220	0.5
Cheese with moulds	31	0.0	164	0.0
Egg product	13	0.0	209	0.0
Mayonnaise	104	0.0	520	0.0
Meat	410	2.4	505	3.8
Poultry	560	0.0	229	6.1
Smoked meat	10	0.0	177	—
Meat product	4331	0.3	3535	0.3
Drinking milk cream	1077	0.0	476	0.6
Yoghurt	1628	0.0	950	0.0
Hard cheese	1097	0.4	1545	5.4
Butter	266	0.0	140	0.0
Milk product	300	0.0	782	0.1
Ready to eat, semi finished product	1038	0.0	167	0.0
Raw cow milk	1444	2.1	860	7.6
Raw goat milk	4	0.0	22	0.0
Raw sheep milk, cheese	146	0.0	732	0.8
Another	191	0.0	832	2.0
TOTAL	**13009**	**0.5**	**13891**	**1.7**

State Veterinary Administration of SR and State Veterinary Institutes

Food based on animal origin belongs to State Veterinary Service of SR. State veterinarians collect samples from farmers, slaughter-houses, dairies, groceries and then they are analysed mainly in State Veterinary Institutes.

The results concerning *L. monocytogenes* monitoring obtained from these laboratories and from State Institute of Veterinary Hygiene and Epidemiology in Trnava are shown in Table 1. It is evident that *L. monocytogenes* was detected as in slaughter animals and raw materials (cattle, pigs, meat, poultry, raw cow and sheep milk) as in certain food (cheese, meat products, drinking milks and creams, other milk products) and also on surfaces in dairies and slaughter houses and in brine baths.

From dairy products the most positive samples were found in hard cheese — 5.4 % in 1999. This type of cheese is made from pasteurised cow milk (usually at 72 °C, for 15—20 seconds) and moreover during the process cheese can be treated with hot water to produce steamed cheese. It is not clear now, whether pasteurisation procedure has not been sufficient to kill *Listeria* in raw milk or cheese has been contaminated from surfaces in dairy or from brine bath. However, hard cheese does not support the multiplication of *L* monocytogenes, and that's way the significance of this contamination is considered as lower risk.

In spite of the fact that listeriosis has appeared in sheeps in Slovakia, *L. monocytogenes* seems to be associated more with raw cow milk than with raw sheep milk. Therefore very low occurrence of *L. monocytogenes*24 in Slovak cheese speciality — soft cheese made also from nonpasteurised sheep milk — "Bryndza" (0.5 % in 1999 and no positive sample in 1998) is not surprising.

Generally, *L monocytogenes* was found slightly more often in 1999 than in 1998. I think it is also consequence of improving the analytical techniques and methods used in monitoring. The Reference Laboratory for Food Microbiology was organised at State Veterinary Institute in Dolný Kubín and it was also well equipped on *L. monocytogenes* detection. There is an agreement that all isolated strains of *L. monocytogenes* are sent here for confirmation.

Table 2: Survey of listeriosis (State Institute of Health of SR, 2000)

Year	Patients	Illness per 100 000 inhabitants
1980	3	0.10
1981	6	0.10
1982	4	0.10
1983	4	0.10
1984	4	0.10
1985	11	0.20
1986	12	0.20
1987	4	0.10
1988	8	0.20
1989	9	0.20
1990	10	0.20
1991	10	0.20
1992	7	0.10
1993	1	0.06
1994	7	0.10
1995	6	0.10
1996	6	0.10
1997	4	0.10
1998	4	0.10
1999	2	0.10

State Institute of Health of SR and State Institutes of Health

State Institutes of Health are executive bodies of Ministry of Health of SR doing health supervision, working with food safety and monitoring illnesses caused by spoiled food in general.

Detection of *L. monocytogenes* in suspected food has been introduced since 1996 as recommendation of FAO/WHO. During the period of 1996—1999 1 712 samples were analysed and 75 strains of *L. monocytogenes* were isolated, the most of them in 1999 — 49 (Figure 1). They were found in steamed cheese, ripening cheese, cheese with moulds, and in meat products, made in Slovakia or imported here. The specialised laboratory on *L. monocytogenes* detection was established at State Institute of Health of SR in Bratislava.

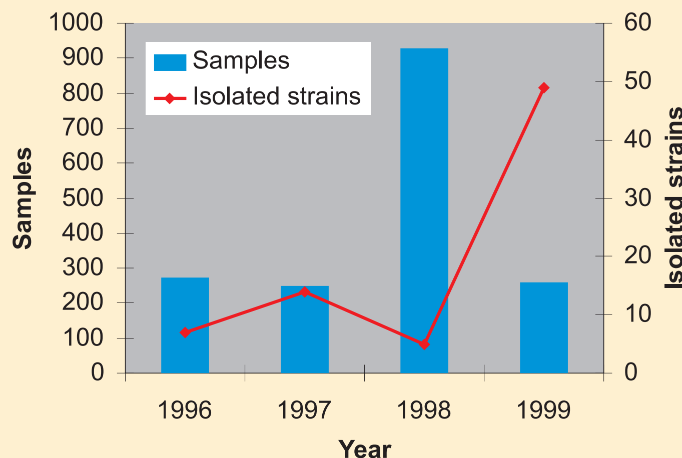

Figure 1: Number of analysed food samples and isolated strains of *L. monocytogenes* (State Institute of Health of SR, 2000)

In addition to *L. monocytogenes* monitoring, the activities of State Institutes of Health are focused on listeriosis diagnosis in suspect patients. Survey of listeriosis during last 20 years is showed in Table 2. The illness per 100 000 inhabitants was very low, with the average about 0.1. The maximum patients was confirmed in 1986 — 12. The National Reference Centre for Listeriosis was held at State Institute of Health in Košice.

Methods and techniques

In Slovakia all standards (also referring to analytical food microbiology) are issued by Slovak Institute of Technical Standardisation as Slovak Technical Standards (STN). In the last years mainly ISO and EN standards have been adopted. In the field of *Listeria* detection, the ISO 10 560:1993 "Milk and milk products — Detection of *Listeria monocytogenes" was* issued in 1999. Another ISO standard the ISO 11 290:1996 "Microbiology of food and animal feeding stuffs -Horizontal method for the detection and enumeration of *Listeria monocytogenes* — Part 1: Detection method" was issued in 2000. The second part of this standard "Part 2: Enumeration method" is under discussion and it is going to be issued soon. However, all accredited laboratories doing *L. monocytogenes* detection were trained on these procedures earlier.

Besides, other cultivation techniques are used, e.g. API-Listeria (bioMerieux); Rapid L. Mono (recently Sanofi Diagnostics Pasteur, now Bio-Rad) to reach results with high quality. The reference laboratories are equipped also with more sophisticated technology, like PCR or anti-bodies. PCR detection of *L. monocytogenes* was developed under other projects and it has been implemented in State Veterinary Institute in Dolný Kubín and in Food Research Institute in Bratislava. Analysis with "mini Vidas„ (bioMerieux) has been available at State Institute of Health of SR in Bratislava and at State Institutes of Health in Banská Bystrica and in Košice since 1999.

In conclusion, rapid methods suitable for *L. monocytogenes* detection play important part of safe food system in Slovakia.

Martin Tomáška

Acknowledgement

I would like to express my gratitude to State Veterinary Administration of SR and State Institute of Health of SR for providing essential information published here.

Foodborne pathogen database

To help ensure the safety of refrigerated foods in the U.S., the U.S. dairy industry has developed a new database that helps identify and characterize various forms of microbial isolates, such as different strains of *Listeria*. Because harmful strains of *L. monocytogenes* can cause outbreaks of food related illness, rapid detection of hazardous foodborne microbes is invaluable in pinpointing the source of contamination. In fact, late in 1998, the research team at Cornell University in Ithaca, N.Y., that developed this database used their knowledge to help curb a serious outbreak of listeriosis by identifying the source of contaminated food in this case, hot dogs.

To date, researchers have compiled information on more than 1,000 strains of the bacteria, in a project funded by America's dairy farmers and managed by Dairy Management Inc. (DMI). Principal coordinator on this project, Kathryn Boor, assistant professor in the department of food science at Cornell University, indicates this database offers outstanding benefits to the scientific and manufacturing communities, in addition to the rapid microbial detection.

First, the database can help processors identify harmful bacteria before food lots are released or sold to the public, and avoid costly recalls and any potential illness.

Second, the characterization of microbial isolates can help the food industry reliably differentiate between pathogenic and non-pathogenic organisms to prevent a false positive identification. In one example, the database helped a northeastern cheese manufacturer salvage its reputation when a consumer identified one of its products as a possible cause of a foodborne illness. Using the database, researchers were able to determine that the company's product was free of any pathogenic organisms and that another food source was the cause of the consumer's illness.

Third, researchers throughout the food industry are welcome to access the database to obtain characteristics of various strains of microbes to determine the most effective processing method to either eliminate undesirable bacterial strains or prevent their growth.

"The database will provide the food industry with the greatest value as it grows with new information," said Boor. "We want to encourage other researchers to use the database and also add to the collective knowledge about *Listeria*, because with more information we will be better able to combat harmful bacteria and increase the already high measure of safety in our food supply chain."

The importance of tools such as the Listeria database can not be overemphasized. In a national radio address delivered this spring, President Clinton stated his renewed commitment to wiping out foodborne illnesses, citing DNA fingerprinting techniques, like this database, as an important safeguard in the nation's food inspection system. The database information can be used to ensure the safety of any type of refrigerated food, including fish, deli meats, cheese and other dairy products.

For more information about the database project, call the DMI technical assistance hotline at 800–248–8829, or log onto the web site at f1www.extraordinarydairy.com.

Report from conference by G. Wyatt

Society for Industrial Microbiology

Food-borne Pathogens 2000 Perspectives and Interventions

April 16—19. 2000
Arlington/Crystal City, VA

Introduction to the Society

What is SIM

The Society for Industrial Microbiology (SIM) is a professional association dedicated to the advancement of microbiological sciences, specifically as applied to industrial materials, processes, products and their associated problems. Its members constitute scientists employed in industry, government and university laboratories.

SIM serves as liaison between the various specialized fields of theoretical and applied microbiology. It promotes the exchange of scientific information through workshops, meetings and publications, in such areas as fermentation processes, bioremediation/biodeterioration, recombinant DNA technology, secondary metabolism, biotransformation, QAQC cosmetic microbiology, the environment and food, among others.

The meeting — a personal view

The meeting opened on the Sunday evening with a keynote speaker, Dr. Arthur Miller from the US Food and Drug Administration (FDA), Washington, who posed the question "What is the food-safety challenge today?". He was obviously speaking from a US perspective, and he described the FDA Food Safety Initiative (FSI) which was introduced by President Clinton, and is now in its third year. The FSI is based on using the principles of *clean*, *separate*, *cook* and *chill* during food production and handling; research priorities are rapid methods for pathogen detection, and anti-microbial techniques.

Particular current problems in the USA are sprouted seeds, and fruit juices (especially apple juice), where *E. coli* O157 is increasingly seen.

Six sessions of scientific talks followed over the next three days.

Session 1, "Food-borne pathogens: Know the enemy", gave overviews of the main organisms of concern, with talks by well-known workers in the field. For example, David Acheson (Boston) described the verotoxigenc *E. coli* and highlighted the on-going spread of VT genes from O157 to other serotypes — this may well have been assisted by the use of DNA-damaging antibiotics (in particular, the fluoroquinolones), which can induce the bacteriophage that carry the genes. Of particular relevance to our project was the talk given by Jeff Farber (Ottawa) on *Listeria monocytogenes*; Jeff expressed the opinion that "*Campylobacter* will die if you look at them but cause much illness; *L. monocytogenes* cannot be killed by a sledgehammer yet causes little illness"! Seriously, he is doing some interesting work on biofilm formation, and has shown that films of certain *L. monocytogenes* strains, especially serotypes 1/2a, can form on stainless steel, a finding of relevance to the food-processing industry.

The second session, entitled "New analysis and detection techniques", was also very relevant to this project. The talks included one by Frances Ligher (Washington) on the use of biosensors for detection of bacteria, viruses and toxins. She is developing fibre-optic biosensors, based on the waveguide principle, using fluorescent-labelled antibodies. In particular, she has produced miniaturised disposable "coupons" containing 4 probes in an array, and associated instrumentation. Other devices include microscope slide waveguides, with 6 × 6 arrays, on which a flow-cell is placed; after binding of the target, image analysis is applied to the slides. A particular highlight of this session for me was the talk by Mansell Griffiths (Guelph), who described the use of bioluminescence in food microbiology. He showed, for example, work with bioluminescent strains of *Salmonella*; here, the use of sophisticated luminometers allowed direct visualisation of luminescent cells inside whole eggs from outside the shell, and colonisation of spleen and gut of mice visualised by luminescence through the gut wall. Some excellent photographs accompanied the talk.

The third session covered microbial response to stress. In a particularly interesting talk, Darrell Bayles (Gainesville) described the impact of food-processing regimes on virulence genes; he showed how stresses introduced by processing and formulation of foods (e.g. heat, osmolarity etc.) can trigger adaptive changes in food-poisoning bacteria, potentially leading to increased virulence of the cells.

The next two sessions covered reduction of food-poisoning by intervention procedures, especially risk reduction on-farm and during processing; particular emphasis was given to the importance of identifying the primary source of the organism, which is generally an ecological niche where it has become established.

The final session covered regulatory aspects of the microbiological safety of foods. Not surprisingly, this was heavily orientated to the USA situation, but during discussions some comparisons were made to the situation in Europe. It was emphasised by an industrial representative (Virginia Scott, Washington) that industry should exchange ideas with government on regulation, not just accept the government viewpoint. She also suggested that HACCP (mandatory in the USA) is causing problems, has become "regulatory HACCP" rather than "food-safety HACCP", and is thus moving away from the original concept; she quoted the saying "The beatings will continue until morale improves" as how this was seen in industry.

There was also an interesting poster session, and here I presented some of the initial work from our project (Wyatt *et al*., 2000), and also a second poster on some of my other work at IFR. A number of rapid detection methods were presented on posters, including some based on binding to antibodies or lectins; nucleic acid based detection methods for pathogens also featured.

Overall, this was a very interesting meeting for me, and the ideas developed within our COPERNICUS project, as presented in the poster, were well received. As I final thought, one speaker quoted President Clinton who, introducing his FSI, said "Food safety is part of our citizen's basic contract with government, it is just that simple" — as scientists, I think we would probably wish that producing solutions to the problem was equally simple!

Reference

G. M. Wyatt, L. Karasová, G. Brett and P. Rauch (2000)
Specific detection of **Listeria monocytogenes** in foods: a novel approach using antibodies to peptides representing virulence proteins.
Poster presented at *Food-borne Pathogens 2000*, Society for Industrial Microbiology, Arlington, VA, April 16th—19th, 2000

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Visit of Andrea Šovčíková and Ludmila Karasová to the Dublin City University, School of Biotechnology, Dublin, ir

Visit of Andrea Šovčíková to the laboratory of Prof. Richard OKennedy took place from 3rd May to 8th July 2000 (half of this stay was supported by the Open Society Foundation) and Ludmila Karasová visited this laboratory from 23rd May to 22nd 1 June 2000.

The main objectives of our visit were:

— familiarisation with laboratory equipment using biosensors
— examination work with biosensors on a chosen model system
— testing of biosensor utilisation efficiency for the INCO COPERNICUS project

Our visit to the School of Biotechnology in Dublin resulted in:

1. familiarisation with biosensors — from the simplest biosensor prototype developed by John Quinn to BIACORE 3000
2. measurement on BIACORE 3000 including simple program creation necessary for its running and evaluation of obtained graphs.
3. testing of different ways of Internalin B immobilisation on chip surface and consecutively binding of antibodies against Internalin B on this surface which is a base for competitive measurements.

Moreover great part of Andrea Šovčíkovás stay in Dublin was focused on familiarisation and measurement of the basal cellular metabolism of A431 carcinoma cells using the cytosensor microphysiometer. This biosensor method which uses global detection mechanisms (proton production and secretion) was compared to the widely used tetrazolium salt assay (MTT test) which is relied on cytotoxicity prediction based on the activity of mitochondrial succinate dehydrogenase.

In conclusion we would like to thank Prof. Richard OKennedy, John Quinn and their collaborators for providing us with this interesting work and for their guidance during our stay in the laboratory at the School of Biotechnology. Furthermore, we would like to extent our appreciation for their constructive discussions, support and help at every step. Also we wish to express our gratitude for the successful realisation of our visit and to the improvement of our knowledge.

Andrea Šovčíková and Ludmila Karasová

Visit of Ludmila Karasová to the Institute of Food Research, Norwich, uk

The aim of my visit that took place from 17th September to 11th November 1999 was training in production of monoclonal antibodies to multiple antigenic peptides (MAPs).

My two-month visit enabled me to get familiar with all stages of production of monoclonal antibodies, such as:

— immunisation;
— fusion of splenocytes with myeloma cells;
— selection of hybridomas;
— culture of particular clones.

I also acquainted myself with monitoring of cell growth and production of specific antibodies by measuring their binding to immobilised antigens. As antigens, I used not only MAPs and virulence proteins but also listeria cells. For this task, it was necessary to cultivate, to inactivate and to count listeria cells. All these operations I learnt here and so it was also very useful for me.

At the same time I tried to develop competitive and sandwich formats of ELISA by using combinations of rabbit antibodies from ICT Prague and unpurified mouse antisera from IFR Norwich.

My visit to the Institute of Food Research in Norwich resulted in:

1. familiarisation with laboratory equipment used to conduct research
2. familiarisation with methods of production, manipulation and monitoring of hybridoma culture
3. knowledge and experience of the scientific team in Norwich handed over to us; this led to improved examination of the production of monoclonal antibodies in the laboratory in ICT Prague.

I would like to stress my very friendly welcome in the Institute, to appreciate the hospitality of the laboratory staff and wonderful working atmosphere within the scientific team. I would like to thank Gary Wyatt and his collaborators for excellent organisation of my visit, for preparation of an interesting work programme and for valuable and stimulating discussions. Also I wish to express my gratitude for the kindness, help and patience I met.

Ludmila Karasová

Recent outbreaks of listeriosis in Ireland

In Ireland, food poisoning statistics arise from notifications by the Health Boards under the Infectious Disease Regulations, 1981. Under the regulations statutory Notifications are made by the attending clinician who is then obliged to notify the Department of Health and Children. But according to the Food safety Authority of Ireland, there is a large variation throughout the country in the investigating and reporting procedures used for identifying cases of food poisoning. In 1998 the Food Safety Authority of Ireland conducted a survey in collaboration with the Public Health Infections Working Group with a view to developing a standard system for investigating and reporting cases of food poisoning throughout the country. With a new and more sophisticated system for investigating and reporting cases of food poisoning, food poisoning cases due to listeria and other bacteria will become more apparent (1273 cases notified in '98 compared to 448 cases in '97), increasing the need for stricter production processes and rapid detection systems.

An investigation into the availability of records and statistical data is very poor in the Republic of Ireland. This contrasts with other nations such as the U.S. and EU member states which keep accurate records of listeriosis and food contamination and such information is made available to the general public.

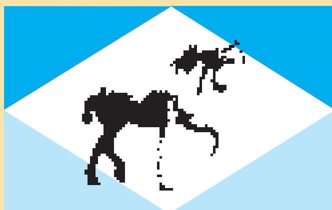
Such records are essential for the implementation of effective preventive measures to reduce incidences of listeriosis. The work undertaken in this project will provide a rapid detection system for the presence of pathogenic listeria. This technology if implemented by the relevant food safety authority and health services would greatly facilitate the recording of both the prevalence of listeria in food and in the environment, giving realistic measure of the dangers of contracting listeriosis. It is important that this information is conveyed between the Public health services, the Food Safety Authority, and the general public occurs. The lack of documented cases in the Republic of Ireland, does not necessarily reflect the true rate of infection. Many cases remain undetected due the similarity of symptoms with the Flu and because the general incidence of food caused by cf1L. monocytogenes remain undiagnosed and recorded as general bacterial food poisoning. The lack of a rapid assay for the detection of *Listeria* spp. may account for the absence of documented cases of human listeriosis.

Paul Leonard

22nd Congress of the Czechoslovak Society for Microbiology

on the topic

HEALTH AND MICROORGANISMS



5th—9th September 2001
to be held in
Košice, Slovakia

Congress section will include:

- subviral agents
- bacterial and mycotic toxins
- malignant transformation and microorganisms
- food and microorganisms
- microorganisms in local immunity
- microorganisms in the generation of the environment
- antibiotic and non-antibiotic trends in the fight with microorganisms
- molecular epidemiology of grave viral infections of humans and agricultural animals
- animal pets and human health

The Conference Secretariat:

Prof. Ivan Mikula, DSc.
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For further information:

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VIth International Conference on Agri-Food Antibodies

3rd—5th October 2001
to be held in
Krystal Hotel, Prague,
Czech Republic

The meeting will bring together researchers with interest in agriculture and food united by the use of polyclonal, monoclonal and recombinant antibodies.

Scientific committee:

Aart van Amerongen, Milan Fránek, Bruce Hammock, Pablo Hernandez, Jan Káš, Roy Jackman, Erwin Maertlbauer, Mike Morgan, Pavel Rauch, Jim Rittenberg, John Skerritt, Petr Skládal, Chris Smith, Larry Stanker, Neale Lowers, Gary Wyatt, Yoshio Ueno

The Conference Secretariat:

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