

13 Seminal plasma signalling in the female reproductive tract. S. A. Robertson*, *The University of Adelaide, Adelaide SA, Australia.*

Providing sperm for conception is generally thought to be the sole male contribution to pregnancy. But this view is now outdated – as well as sperm, semen contains potent signalling molecules that influence female reproductive physiology to improve the chances of pregnancy success. Cytokines and prostaglandins secreted by seminal vesicle and prostate glands bind to receptors on target cells in the female reproductive tract, activating changes in gene expression leading to modifications in the cellular composition, structure and function of the cervix and uterus. The consequences are increased sperm survival, ‘conditioning’ of the female immune response to tolerate the conceptus, and molecular and cellular changes in the endometrium that facilitate embryo development and implantation. Male-female tract signalling occurs in rodents, livestock animals and all other mammals so far studied including humans. The key active factors in seminal plasma are identified as members of the transforming growth factor- β family.

Experiments in rodents show that disruption of seminal plasma priming causes fetal growth retardation and changes in placental structure, with long-term consequences for growth of the neonate. Recent studies indicate a similar function for seminal plasma in the pig. In gilts, seminal plasma elicits an endometrial response with recruitment of inflammatory leukocytes and induction of pro-inflammatory cytokines and cyclo-oxygenase-2. The consequences persist through the pre-implantation period with altered leukocyte populations and cytokine parameters seen for at least 9 days. Exposure to semen also alters the dynamics in pre-implantation embryo development with an increase in the number of embryos and in their viability. Furthermore seminal plasma influences the timing of ovulation, corpus luteum development and progesterone synthesis. A better understanding of seminal plasma signalling may lead to new treatment products and management protocols to ensure maximal fertility and reduce embryo mortality in livestock animals.

Key Words: Seminal plasma, Cytokines, Pregnancy

Dairy Foods: Advances in Cultured Foods

14 Historical overview of lactic cultures. R. Sellars*, *R. L. Sellars and Associates, Waukesha, WI.*

A historic overview of the culture problems in the cheese industry that initiated the need for lactic culture research will be briefly presented. This led to the first commercialization of lactic acid producing starter-cultures used by the European and North American cheese industries. Interactions between the commercial starter houses, academia, and the cheese industry that led to the development of the current service oriented industry will be highlighted. The search for suitable cultures, protective media, plant sanitation procedures and starter rooms that reduce bacteriophage infections will also be discussed. The most important commercial milestones of starter-culture developments by commercial companies from 1878 to present and the strategies to produce flavorful, consistent hard cheeses will also be presented.

Key Words: Lactic culture, Cheese, Starter

15 Non-starter lactic acid bacteria. T. M. Cogan* and T. P. Beresford, *Moorepark Food Research Centre Teagasc, Fermoy, Ireland.*

In cultured dairy products, non-starter lactic acid bacteria (NSLAB) are only important in ripening and ripened cheeses. They mainly comprise facultatively homofermentative lactobacilli, e.g., *Lactobacillus casei*, *Lb. paracasei*, *Lb. curvatus* and *Lb. plantarum*, although pediococci and enterococci are found in lower numbers in some cheeses. Some obligate heterofermentative species, e.g., *Lb. brevis* are occasionally found. During ripening the NSLAB grow from low numbers of c. 10^2 to 10^8 cfu/g over several weeks or months depending on the cheese and its ripening temperature. NSLAB require an energy source for growth but lactose has been metabolised completely to lactate by the time exponential growth of NSLAB occurs. Citrate, arginine, sugars in lysed starter cells and in the milk fat globule membrane have been suggested as energy sources in cheese. The chromosome of at least one strain has been completely sequenced. Generally it is believed that their source is post pasteurisation contamination from equipment and air. However, many strains resist pasteurisation implying that raw milk is also a major source. The effect of NSLAB on cheese flavour has

been studied for over 120 years with variable results. More recent studies have generally shown a positive effect of selected strains on cheese flavour formation. During ripening, they are responsible for converting L lactate to D lactate and cause development of white spots on aged Cheddar cheese due to calcium-D-lactate formation. Many NSLAB metabolise citrate to formate and acetoin; they also metabolise amino acids, especially methionine to potent S containing flavour compounds like methanethiol and its degradation compounds, as well as various S containing esters. These and other aspects of NSLAB will be discussed in the presentation.

Key Words: NSLAB, Cheese, Lactobacilli

16 Insights from genomic studies on dairy lactic acid bacteria. J. L. Steele*, *University of Wisconsin, Madison.*

The genome of lactic acid bacteria (LAB) contains both plasmid and chromosomal DNA. The characterization of plasmids in lactic acid bacteria has been an ongoing area of study for the past thirty years. Characterization of LAB chromosomes begun in the early 1970s, however the most exciting developments in LAB genomics are now being fueled by nucleotide sequence information for complete chromosomes. Currently, the genome sequence is known or is being determined for more than twenty LAB. The value of genome sequence information for dairy-related LAB cannot be overstated. The availability of genomic sequences allows researchers to rapidly ascertain the genetic potential of an organism. For example, investigations into the proteolytic enzyme system of *Lactobacillus helveticus* CNRZ32 over a twelve year period had resulted in the identification of twelve genes encoding components of this system. However, within three months of obtaining a draft genome sequence of this organism, an additional thirteen genes encoding proteolytic enzymes were identified. Additionally, the availability of multiple genome sequences within a species allows for the study of strain specific traits. For example, a comparison of the complete genome sequence of two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* identified regions involved in bacteriophage resistance, a trait known to vary from strain to strain. The availability of genome sequences also allows studies to follow

global gene regulation via DNA microarrays. A major strength of this technology is that it provides a non-biased global view of an organism's transcriptional response to an environment of interest. This unbiased holistic view consistently yields unexpected observations that ultimately lead to the identification of genes with critical functions in the physiological system of interest. An example of this outcome was the identification of expression of a pathway for the utilization of serine-phosphate during growth of *Lb. helveticus* CNRZ32 in milk. Access to genomic information has provided researchers with unprecedented power to elucidate mechanisms by which LAB have adapted to milk and cheese environments.

Key Words: Genomics, Lactic acid bacteria, Sequence

17 Engineering culture attributes. J. Broadbent*, *Utah State University, Logan.*

Metabolic engineering of cells is founded upon the power to establish cellular and molecular functions via DNA manipulation. Today, the ability to genetically manipulate or 'engineer' animals, plants, and microorganisms to manufacture, modify, or improve products or processes has blossomed into a multibillion dollar enterprise that has revolutionized the pharmaceutical, chemical, and agricultural industries. Many of the most exciting and successful applications involve microbial products. While use of recombinant DNA (rDNA)-derived microbial products in agricultural and food systems is commonplace, a similar situation does not apply to the use of live, rDNA-containing microbial cells. When we consider potential applications for genetically modified dairy cultures, it is important to recognize a few basic principles: 1) dairy starter technology can be traced to the late 19th century, and their long history of safe application in human food provides dairy starter bacteria with GRAS status; 2) existing knowledge of starter genetics and physiology has already identified clear strategies to improve the industrial performance of these cells; and 3) in some cases, these improvements can be effected by means other than rDNA technology. Examples of genetic improvements that satisfy these criteria and have already been implemented by industry involve introduction of phage-resistance plasmids and the selection for enhanced diacetyl production in lactococci. Moreover, rDNA experiments with starter bacteria in research laboratories has fueled important advancements in culture technology. This is because rDNA methods permit construction of isogenic cells that differ from their wild-type strain only by the action of a single polypeptide. By comparing the wild-type culture to its isogenic derivative, the role of that polypeptide in a particular process can often be unequivocally established. From this knowledge, dairy technologists can utilize strain selection or screening methods to identify starter bacteria that already possess the trait of interest.

Key Words: Lactic acid bacteria, Starter culture

18 Use of bacteriophage peptides as vectors or blockers to receptors on lactic cell membranes. C. Hicks*, *University of Kentucky, Lexington.*

Bacteriophage (phage) peptides hydrolyzed (.01% ficin at 31°C) from *Lactococcus lactis* ssp *lactis* c2 phage were tested to determine their effectiveness in blocking c2 phage from attacking *L. lactis* C2 host. Initial results showed that c2 peptides had only a minor inhibitory affect on culture growth. Heat treatments =111°C were effective in

killing any contaminating c2 phage that were present in the purified peptides without affecting peptide activity. Extended cell growth times were observed when c2 phage peptides (2.5%) were added to growth medium (M17) where the culture was infected with c2 phage (10^2 , 10^4 and 10^8 pfu/ml). When phage infections were at 10^2 pfu/ml, *L. lactis* C2 culture could grow to the stationary phase when 2.5% c2 phage peptide was present. When *L. lactis*C2 culture was grown in 2% c2 phage peptide and used to inoculate milk which was infected with 10^3 pfu/ml of c2 phage, acid developed faster than when the culture was added to milk containing 2% c2 peptide. Although some protection was observed when c2 peptide was added directly to the cheese milk, c2 peptide was most effective when added to the culture medium. In other experiments peptides were prepared from *L. plantarum* yit0068 phage and *L. Lactis* ssp *lactis* ml3 phage. These peptides were tested with *L. plantarum* and *L. lactis* C2 hosts that were infected with their specific phage. Growth times of *L. plantarum* were extended up to 25% as peptide (yit0068) concentration increased in the growth medium. When *L. plantarum* was grown in medium containing 4% yit0068 peptide and infected with 10^2 pfu/ml (yit0068 phage), the culture reached the stationary phase without lysing. Peptides derived from ml3 and yit0068 phage inhibited *L. lactis* C2 culture growth and did not inhibit phage proliferation equal to the c2 phage peptides.

Key Words: Bacteriophage, Culture, Cheese

19 Media development for selective enumeration of lactic acid bacteria. N. P. Shah*, *Victoria University, Melbourne, Victoria, Australia.*

Because of the potential health benefits, probiotic organisms such as *Lactobacillus acidophilus*, *Bifidobacterium* spp., and *L. casei* are increasingly incorporated into dairy foods. Viability of probiotic bacteria is important in order to provide health benefits. In order to assess viability and survival of probiotic bacteria, it is important to have a working method for selective enumeration of these probiotic organisms. Several media for selective enumeration of *L. acidophilus*, *Bifidobacterium* spp., and *L. casei* have previously been proposed. However, most of these methods are based on pure cultures of these organisms and may not be suitable for selective enumeration of probiotic organisms in the presence of other probiotic organisms and yogurt culture organisms (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*). Further, differences exist among the strains of the same species with respect to sugar fermentation characteristics and tolerance of low pH, and bile. A selective medium *L. casei* (LC) agar has been developed by Ravula and Shah for enumeration of *L. casei* populations from commercial yogurts and fermented milk drinks. MRS-salicin or MRS-sorbitol agar can be used for selective enumeration of *L. acidophilus* provided *L. casei* is not added into the product. However, if *L. casei* is added to the product, then MRS-sorbitol agar or MRS-salicin agar can be used to obtain counts of *L. acidophilus* and *L. casei*, and LC agar can be used to obtain a total count of *L. casei*. The counts of *L. casei* can be subtracted from the total population of *L. acidophilus* and *L. casei* enumerated using MRS-salicin or MRS-sorbitol agar. *Bifidobacterium* can be enumerated on MRS-NNLP agar.

Key Words: Probiotic, Media development

20 Probiotics and health: Their potential role in modulation of immune function. Z. Ustunol*, *Michigan State University, East Lansing.*

About a century ago, Metchnikoff wrote in his book 'The Prologation of Life' that consumption of fermented dairy products by lactic acid bacteria provided in improved health and longer life. Today, an increasing number of health foods, functional foods as well as pharmaceutical preparations are promoted with health claims based on the probiotic characteristic of some of these bacteria. It is widely accepted that the gastrointestinal (GI) microflora play an important role in the health of the host and possess immunomodulating capacity. Probiotic ingestion is thought to alter the GI microflora by providing bacterial cells to this ecosystem and have been suggested as potential candidates for immune modulation. Various studies have been conducted on the effect of probiotic bacteria on immune function. However, most often interpretation of the findings has been inconclusive or conflicting in the absence of clear mechanistic data. It is thought that the mechanism by which probiotics influence the immune system

may relate to their ability to differentially modulate expression of cytokines and co-stimulatory molecules. More recently, probiotics have been reported to modulate innate and acquired immune control and contribute to a more finely tuned T helper 1 (Th1) and Th2 immune responses. Activation of surface receptors designated as Toll-like receptors (TLRs) by bacterial components, are believed to be key for regulation of immune response and to mediate a link between innate and adaptive immunity. Role of probiotics in the linking of innate and adaptive immune function and their capacity to favor Th1 or Th2 immune responses may have significant implications for inflammatory diseases. This presentation will focus on the most current information on immune modulation by probiotic bacteria. Elucidation of the mechanisms by which intestinal microorganisms, and ingested probiotics modulate immune function may facilitate implementation of therapeutic probiotic dairy products that are individually tailored for immunoregulatory properties.

Key Words: Probiotics, Immune modulation, Th1, Th2, TLR