460 The genetic basis for diversity in exopolysaccharide structure and production. G LaPointe*[1], [1]*STELA Dairy Research Centre*.

The functional properties of polysaccharides are related to their charge, molecular mass and sugar composition. The structure of the repeating units of bacterial heteropolysaccharides is determined by the action of glycosyltransferase enzymes with specific substrates, the sugar nucleotide precursors. Modifications include such reactions as acetylation or pyruvylation, and the addition of phosphate or sulphate substituents. The length of the polymers produced is controlled by a complex polymerization mechanism. Gene transfer and recombination events also contribute to the variety of polysaccharide structures synthesized by lactic acid bacteria. Mapping the number and type of glycosyltransferase genes present has thus been useful in predicting the structure of the repeating unit. Physiological studies have revealed the effect of such factors as temperature, pH, oxygen tension and carbon/nitrogen ratio on the availability of the sugar nucleotide precursors and thus on EPS production. However, the regulation of EPS biosynthesis is still very poorly understood. Numerous regulatory circuits require reversible phosphorylation events. Kinase and phosphatase activities have recently been shown to modulate polysaccharide production at the polymerization level. In effect, the genes coding for these activities are conserved within many *eps* and *cps* operons, suggesting a common biosynthetic mechanism. More knowledge of the genetics and biochemistry of EPS biosynthesis is necessary in order to be able to successfully engineer polysaccharide properties by modifying composition and chain length. The diversity in enzymatic capability of lactic acid bacteria will thus facilitate the design of novel polysaccharides for food and pharmaceutical applications.

Key Words: Exopolysaccharide biosynthesis, Glycosyltransferases, Regulation

461 Structure determination of exopolysaccharides from lactic acid bacteria. Marie-Rose Van Calsteren*, *Food Research and Development Centre, Agriculture and Agri-Food Canada*.

Exopolysaccharides (EPS) are exocellular microbial polysaccharides. To date, EPS produced by lactic acid bacteria (LAB) have received increasing interest, mainly because of their generally recognized as safe (GRAS) status, their rheological properties in food, and their potential health-beneficial properties. Most EPS from LAB are heteropolysaccharides. Their structure consists of a repeating unit containing three to seven sugar residues, combinations of D-Glc, D-Gal, L-Rha, D-GlcNAc, D-GalNAc and D-GlcA. Each hexose could adopt the pyranose or furanose ring configuration, and be linked with the α or β anomeric configuration to other residues at several possible positions. Furthermore, substituents, such as acetate, phosphate, glycerol or pyruvate, could be present. Differences in the properties of EPS are due to the large number of possible configurations and linkages. Hence, the study of the structure of EPS is crucial to understand their physicochemical and biological properties, and for the exploitation of EPS-producing LAB in industrial or medical applications. Several chemical and physical techniques are used to determine the primary structure of EPS. Chemical degradation and derivatization combined with chromatographic methods, often coupled to mass spectrometry (MS), are used to determine the sugar composition, together with the absolute configuration, their positions of substitution, and the substituent composition. Nuclear magnetic resonance (NMR), in particular two-dimensional ^{1}H and ^{13}C NMR, is the most powerful technique to obtain information on the nature and configuration of sugar residues, their interconnectivity, and the nature and location of substituents, to ultimately determine the sequence of the repeating unit. Sometimes, chemical or enzymatic fractionation of the polysaccharide is required to produce smaller fragments, more easily analyzed by NMR or MS. Examples of the application of these methods to the structure elucidation of EPS from several species of LAB will be presented.

Key Words: Heteropolysaccharides, Sequence of the Repeating Unit, Nuclear Magnetic Resonance (NMR)

462 Applications of EPS production by LAB. C. J. Oberg*[1], J. R. Broadbent[2], and D. J. McMahon[2], [1]*Weber State University, Ogden, Utah*, [2]*Utah State University, Logan, Utah*.

Many strains of dairy lactic acid bacteria (LAB) manufacture extracellular polysaccharides (EPS). In nature, bacterial EPS fulfills a variety of diverse functions including cell protection, adhesion of bacteria to solid surfaces, and participate in cell-cell interactions. Incorporation of EPS or EPS-producing (EPS+) cultures in dairy foods can provide viscosifying, stabilizing, and water-binding functions. EPS also contributes to the mouth-feel, texture, and taste perception of fermented dairy products. EPS may even play a role in the probiotic activity of certain LAB. Milk fermented with EPS+ dairy LAB generally develops a ropy or viscous texture, and EPS+ strains of LAB are widely used in yogurt manufacture to enhance viscosity and reduce syneresis. Besides yogurt, other dairy products where LAB EPS has been shown to affect product quality include sour cream and traditional fermented milks in Nordic countries. More recently, researchers have shown EPS+ LAB can enhance the functional properties of cheese. Because LAB EPS have excellent water-binding properties and moisture retention is vital to functionality in low fat cheese, the use of an EPS+ starter has been shown to improve the moisture and melt properties of low fat Mozzarella cheese. As the genetics and physiology of LAB EPS continue to be better understood, further applications inside and outside the realm of dairy products will occur.

Key Words: Exopolysaccharide, Lactic acid bacteria, Applications

463 Visualization of bacterial exopolysaccharide in dairy products using confocal laser scanning microscopy. J F Frank*[1] and A N Hassan[1], [1]*Department of Food Science and Technology, University of Georgia*.

Understanding the role exopolysaccharide (EPS) produced by lactic acid bacteria plays in the microstructure and physical properties of dairy products has been difficult because of the inability to visualize EPS within product structures. EPS produced in dairy products is highly hydrated and its structure collapses upon dehydration leaving void areas. Void areas in samples observed using scanning electron microscopy may originate with water, EPS, or air, making interpretation of micrographs difficult. Confocal laser scanning microscopy (CSLM) allows observation of fully hydrated samples which preserves the EPS structural component. Capsular EPS of lactic acid bacteria in milk can be visualized by using CSLM in reflectance mode, as the casein micelles reflect light and are excluded by the capsule, resulting in a clear zone surrounding the bacterial cell. Ropy (slime) EPS can be visualized by CSLM after staining with fluorescent-labelled lectins. Selection of lectins for this purpose is partially by trail and error, as one lectin type does not bind to the EPS of all strains. We visualized ropy EPS in yogurt and feta cheese by staining samples with wheat germ agglutinin labeled with Alexa fluor 488 or concanavalin A 488. EPS was observed to fill pores in the casein structure. Stirring yogurt made with ropy culture produced longer and larger strands of EPS. This technique should lead to improved understanding of the role of EPS in dairy product stucture and function.

Key Words: exocellular polysaccharide, confocal scanning laser microscopy, lectin

464 Does EPS protect LAB against phages? S. Moineau*, D. Tremblay, and H. Deveau, *Universite Laval*.

Virulent phages are the most significant cause of fermentation failures in the dairy industry worldwide. It has been proposed that the production of exopolysaccharides (EPS) by lactic acid bacteria (LAB) may protect the starter culture against phage infection. However, phage-sensitive and phage-insensitive LAB strains that produce EPS are currently used by the dairy industry. Thus, the involvement of EPS in the phage infection process of LAB is still unclear. Recent data from our laboratory indicated that for *Lactococcus lactis* and for *Streptococcus thermophilus*: i) phages infecting eps^{+} strains are not fundamentally distinct from those infecting eps^{-} strains; ii) EPS are not necessary for the phage infection process; iii) in few cases, loosely bound EPS may provide, at best, a very weak protection against phage infection by

partially hindering the phage receptors on the cell surface. Moreover, it is possible that the production of EPS increases the viscosity of the medium and perhaps, this molecular crowding slightly affect the spread of the phage infection. It remains to be seen if the sugar composition or the structure of some EPS produced by LAB may be involved in phage sensitivity or insensitivity. Nonetheless, it is now clear from the above evidences that we should not solely rely on the EPS production to protect starter cultures against phages.

Key Words: Exopolysaccharide (EPS), Bacteriophage, Lactic acid bacteria (LAB)

Physiology
Basic Mechanisms Regulating Anovulatory States

465 Neuroendocrine mechanisms underlying seasonal breeding in the ewe. RL Goodman*[1], GM Anderson[1], VL Adams[1], SL Hardy[1], JM Connors[1], and MN Lehman[2], [1]*West Virginia University,* [2]*University of Cincinnati.*

It is now clear that an increase in response to the negative feedback action of estradiol (E) is responsible for the inhibition of ovarian function in anestrous ewes. In early work, we demonstrated that A15 dopaminergic (DA) neurons play a key role in this response. These neurons mediate E negative feedback in anestrus and their response to E varies seasonally. Because A15 cells do not contain estrogen receptors (ER), other neurons most likely provide information on E levels to them in anestrus. In more recent work, we have focused on the E-responsive component of this circuitry and have identified two important areas: the ventromedial preoptic area (vmPOA) and retrochiasmatic area (RCh). ER-positive cells in both areas project to the A15, and local administration of E to either area inhibits LH secretion in anestrus, but not during the breeding season. Furthermore, the inhibition of LH in anestrus can be overcome by a DA-receptor antagonist. We thus postulated that the neural circuit mediating E negative feedback in anestrus includes E-responsive perikarya in the vmPOA and RCh that project to the A15 and stimulate these DA neurons, which in turn inhibit GnRH release. This hypothesis raises the possibility that structural changes within this circuit may contribute to the seasonal alterations in response to E negative feedback. Therefore, we tested if there is a seasonal variation in synaptic input to A15 perikarya using dual immunocytochemistry to stain for synaptic varicosities (synapsin I) and DA perikarya (tyrosine hydroxylase). Confocal microscopic analysis indicated a significant increase in synaptic close contacts on A15 dendrites in anestrous ewes. This increase in synaptic input correlated with a significant increase in the dendritic arborization of these neurons. Thus seasonal morphological changes within the neural system mediating E negative feedback may well play an important role in the mechanisms responsible for seasonal breeding in the ewe. Supported by NIH HD-17864

Key Words: Seasonal breeding, estrogen negative feedback, sheep

466 Nutrition and suckling mediated anovulation in beef cattle. R.P. Wettemann*, C.A. Lents, N.H. Ciccioli, F.J. White, and I. Rubio, *Oklahoma Agricultural Experiment Station, Stillwater.*

Nutrient intake, body energy reserves, and suckling are major regulators of reproductive performance of beef cows. Inadequate body energy reserves at parturition increase the interval to first estrus and ovulation, and postpartum nutrient intake can influence length of the interval in cows with thin to moderate body condition (BCS). Suckling can increase the postpartum anestrous interval in thin cows but has little effect on mature cows with adequate body energy reserves. Reduced nutrient intake can delay the onset of puberty and cause cessation of estrous cycles. The objective of this presentation is to evaluate signals by which nutrient intake and body energy reserves may regulate ovarian function. Nutritional restriction causes decreased secretion of LH, reduced follicular growth, and decrease concentrations of estradiol in plasma. Pituitary concentrations of LH were reduced and concentrations of FSH were greater in nutritionally induced anovulatory cows that were ovariectomized compared with proestrous cows. Acute energy restriction of postpubertal heifers resulted in decreased IGF-I in plasma, inhibition of the proestrus increase in estradiol and the ovulatory surge of LH, and anovulation. In addition to direct and indirect effects of decreased energy intake on the hypothalamus and pituitary, nutrition may influence ovarian function. Metabolic signals such as insulin, IGF-I, and leptin may regulate functions of the pituitary and ovary. Concentrations of IGF-I in plasma during late gestation are correlated ($r = 0.33$; $P < 0.01$) with BCS, and concentrations of IGF-I and leptin in plasma are greater in postpartum cows that have increased energy intake. Concentrations of IGF-I in plasma increase preceding ovulation when nutritionally induced anovulatory cows are realimentated. Nutrient intake and BCS alter metabolic signals at the hypothalamus and pituitary that control secretion of LH, and reduced stimulation by metabolic signal at the ovary may compromise the ability of follicles to respond to gonadotropins.

Key Words: Anovulation, Nutrition, Beef Cattle

467 Nitric oxide and the ovary. Carlo Tamanini*, Giuseppina Basini, and Francesca Grasselli, *Dip. Prod. Anim., Biotec. Vet., Qual. Sic. Alim., University of Parma-Italy.*

Nitric oxide (NO) is synthesized from L-arginine by NO synthase (NOS), an enzyme with three isoforms; two of them, neuronal and endothelial (n and eNOS) are constitutive, while the third one, iNOS, is inducible. NO is effective in mediating multiple biological effects, at least in part through the activation of soluble guanylate cyclase (cGMP); among these, smooth muscle cell tone, platelet aggregation and adhesion, cell growth, apoptosis and neurotransmission. Being that these mechanisms are associated with the pathophysiology of several reproductive processes, it became clear that NO could play a key role in reproduction. Apart from its effects through the modulation of LHRH release, NO has been proven to act directly at the ovarian level, where it has been demonstrated to be produced by the vasculature and neurons as well as by various cell types, including granulosa, theca and luteal cells; its production is modulated by several hormones (P4, LH, FSH and hCG) and cytokines which interfere with either eNOS or iNOS expression and activity. Experiments performed with NO donors and/or NO synthase inhibitors have demonstrated that NO reduces apoptosis and inhibits both E2 and P4 production by granulosa cells (at least in part via cGMP). NO is possibly involved in follicle growth. In fact, it is a potent mitogen in the presence of basic fibroblast growth factor (bFGF), it increases the receptors for epidermal growth factor on granulosa cells and, as mentioned above, it regulates the programmed cell death (which is an important part of folliculogenesis). The gonadotropin-stimulated eNOS and iNOS expression as well as the inhibition of ovulation by NOS inhibitors suggest that NO participates in the ovulatory process. After ovulation, iNOS is expressed in luteal cells but its activity diminishes with the corpus luteum development; during the luteolysis phase NO stimulates PGF2α synthesis while reducing P4 secretion. The overall information provides convincing evidence that NO plays a critical role in the ovarian physiology with regard to follicle growth, ovulation and corpus luteum function, even if its clinical implications have not been clarified yet.

Key Words: Folliculogenesis, Ovulation, Corpus luteum