Adamo, R., R. Saksena, et al. (2005). "Synthesis of the beta anomer of the spacer-equipped tetrasaccharide side chain of the major glycoprotein of the Bacillus anthracis exosporium." Carbohydr Res **340**(17): 2579-82.

The beta glycoside of the tetrasaccharide sequence beta-Ant-(1-->3)-alpha-l-Rhap-(1-->3)-alpha-l-Rhap-(1-->2)-l-Rhap, whose aglycon allows conjugation to proteins, was synthesized for the first time. A stepwise synthetic approach was applied with thioglycosides as glycosyl donors, and the beta anomer of the compound was obtained equipped with a spacer group whose further transformation allows conjugation to suitable carriers. To synthesize the beta-anthrosyl linkage with high stereoselectivity, a linker-equipped rhamnotriose derivative was glycosylated with ethyl 4-azido-3-O-benzyl-2-O-bromoacetyl-4,6-dideoxy-1-thio-beta-d-glucopyranosi de. Further functionalization of the tetrasaccharide thus obtained, followed by deprotection, gave the target substance.

Albers, S. V., M. G. Elferink, et al. (1999). "Glucose transport in the extremely thermoacidophilic Sulfolobus solfataricus involves a high-affinity membrane-integrated binding protein." J Bacteriol **181**(14): 4285-91.

The archaeon Sulfolobus solfataricus grows optimally at 80 degrees C and pH 2.5 to 3.5 on carbon sources such as yeast extracts, tryptone, and various sugars. Cells rapidly accumulate glucose. This transport activity involves a membrane-bound glucose-binding protein that interacts with its substrate with very high affinity (Kd of 0. 43 microM) and retains high glucose affinity at very low pH values (as low as pH 0.6). The binding protein was extracted with detergent and purified to homogeneity as a 65-kDa glycoprotein. The gene coding for the binding protein was identified in the S. solfataricus P2 genome by means of the amino-terminal amino acid sequence of the purified protein. Sequence analysis suggests that the protein is anchored to the membrane via an amino-terminal transmembrane segment. Neighboring genes encode two membrane proteins and an ATP-binding subunit that are transcribed in the reverse direction, whereas a homologous gene cluster in Pyrococcus horikoshii OT3 was found to be organized in an operon. These data indicate that S. solfataricus utilizes a binding-protein-dependent ATP-binding cassette transporter for the uptake of glucose.

Altman, E., J. R. Brisson, et al. (1992). "Structure of the glycan chain from the surface layer glycoprotein of Clostridium thermohydrosulfuricum L77-66." Biochim Biophys Acta **1117**(1): 71-7.

The thermophilic eubacterium Clostridium thermohydrosulfuricum L77-66 is covered by a crystalline surface layer composed of identical glycoprotein subunits which are arranged in a hexagonal lattice with centre-to-centre spacings of approx. 14.3 nm. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of cell wall preparations showed the presence of several broadened, carbohydrate-containing bands in a molecular mass range of 90 to 200 kDa. A total carbohydrate content of approx. 14% was determined in the purified surface layer glycoprotein. Chemical deglycosylation of this material by

trifluoromethanesulfonic acid resulted in the disappearance of the complex banding pattern. Only a single band with a molecular mass of 82 kDa remained visible upon Coomassie staining. After proteolytic digestion of the surface layer glycoprotein a single glycopeptide fraction with an apparent molecular mass of approx. 25 kDa was obtained by gel filtration. Composition analysis, methylation, periodate oxidation and a combination of homonuclear and 1H-detected heteronuclear shift-correlated nuclear magnetic resonance experiments established the following structure for the glycan chain of the surface layer glycoprotein.

Altman, E., J. R. Brisson, et al. (1990). "Chemical characterization of the regularly arranged surface layer glycoprotein of Clostridium thermosaccharolyticum D120-70." Eur J Biochem **188**(1): 73-82.

Clostridium thermosaccharolyticum D120-70 possesses as its outermost cell envelope layer a square-arranged array of glycoprotein molecules. SDS/polyacrylamide gel electrophoresis of the purified surface layer showed a broadened band in the molecular mass range of about 115 kDa which, upon periodic acid/Schiff staining, gave a positive reaction. After proteolytic degradation of this material, two glycopeptide fractions were obtained. One- and two-dimensional nuclear magnetic resonance studies, together with methylation analysis and periodate oxidation, were used to determine the structures of the polysaccharide portions of these glycopeptides. The combined chemical and spectroscopic evidence suggests the following structures: (formula; see text).

Altman, E., J. R. Brisson, et al. (1991). "Structure of the glycan chain from the surface layer glycoprotein of Bacillus alvei CCM 2051." Biochem Cell Biol **69**(1): 72-8.

The cell surface of the mesophilic eubacterium Bacillus alvei CCM 2051 is covered by an oblique arranged surface layer glycoprotein. The subunits revealed by sodium dodecyl sulfate - polyacrylamide gel electrophoresis were distinct bands of molecular masses 140,000, 128,000, and 127,000. Proteolytic degradation of the purified S-layer glycoprotein yielded a single glycopeptide fraction with an apparent molecular mass of ca. 25,000. Methylation analysis in conjunction with two-dimensional nuclear magnetic resonance experiments at 500 MHz established the branched trisaccharide (formula; see text) as the repeating unit for this glycan chain.

Altman, E., C. Schaffer, et al. (1995). "Characterization of the glycan structure of a major glycopeptide from the surface layer glycoprotein of Clostridium thermosaccharolyticum E207-71." Eur J Biochem **229**(1): 308-15.

The squarely arranged surface layer (S-layer) glycoprotein of Clostridium thermosaccharolyticum E207-71 was isolated from bacterial cells which were grown under defined culture conditions. By sodium dodecyl sulfate polyacrylamide gel electrophoresis, the S-layer showed a series of distinct bands with apparent molecular masses in the range 83-210 kDa. Upon deglycosylation by trifluoromethanesulfonic acid, only the single band at 83 kDa remained unchanged. After pronase digestion of the intact S-layer glycoprotein, the

degradation products were isolated by gel-permeation chromatography, cation-exchange chromatography and isoelectric focusing. Three main fractions and an amino sugar containing minor fraction were obtained. The main fractions, which showed identical carbohydrate compositions, were further purified by reverse-phase chromatography and characterized by monosaccharide analysis, Smith degradation, methylation analysis, and one-dimensional and two-dimensional nuclear magnetic resonance spectroscopy. The combined chemical and spectroscopical evidence suggest the following glycan structure for the main fractions: [Sequence: See text]

Altman, E., C. Schaffer, et al. (1996). "Isolation and characterization of an amino sugar-rich glycopeptide from the surface layer glycoprotein of Thermoanaerobacterium thermosaccharolyticum E207-71." Carbohydr Res **295**: 245-53.

Bader, J. A., P. H. Klesius, et al. (1997). "Comparison of whole-cell antigens of pressure- and formalin-killed Flexibacter columnaris from channel catfish (Ictalurus punctatus)." Am J Vet Res **58**(9): 985-8.

OBJECTIVE: To identify and compare immunodominant antigens in whole-cell lysates of pressure- and formalin-killed Flexibacter columnaris. ANIMALS: Sera from naturally infected and vaccinated channel catfish. PROCEDURES: Whole-cell lysates of pressure- and formalin-killed F columnaris were compared, and antigens were isolated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The antigens were identified by staining, western blotting, and specific monoclonal antibodies to glycoproteins. Western blotting was performed, using sera from channel catfish (Ictalurus punctatus) with naturally acquired F columnaris infection and sera from channel catfish vaccinated with an experimental prototype F columnaris vaccine. RESULTS: Whole-cell lysates of pressure and formalin-killed F columnaris shared 4 proteins: 100, 80, 66, and 60 kd. The 60-kd antigen was a glycoprotein. Western blotting, using sera from naturally infected channel catfish, revealed the same proteins for pressure- and formalin-killed F columnaris. Sera from vaccinated fish reacted only to pressure-killed lysate antigens. CONCLUSIONS: Pressure- and formalin-killed F columnaris whole-cell lysates share 100-, 80-, 66-, and 60-kd proteins and are recognized by antibodies from naturally infected catfish and those vaccinated with formalin-killed F columnaris. Formalin treatment modifies or inactivates the 60-kd protein antigens, rendering them unrecognizable to antibodies from channel catfish naturally infected with F columnaris, suggesting that formalin-killed F columnaris may not be suitable for use as a bacterin against columnaris disease.

Bedouet, L., F. Arnold, et al. (1998). "Evidence for an heterogeneous glycosylation of the Clostridium tyrobutyricum ATCC 25755 flagellin." Microbios **94**(379): 183-92.

Glycosylation analysis of the flagellin from the Gram-positive species Clostridium tyrobutyricum has been supplemented. Amino acid analysis of the glycopeptides obtained after pronase digestion of flagellin indicated that O-glycosylation which was previously demonstrated after nonreductive beta-elimination, probably

occurred via the hydroxyl group of serine. Otherwise, beta-elimination partly deglycosylated flagellin. After this treatment carbohydrates were still linked to protein as shown by a digoxigenin-hydrazide labelling. Therefore, in addition to linkages via serine, alkaline resistant linkages exist on the flagellin and some glycans may be linked to the protein core via the amide nitrogen of asparagine or via the hydroxyl group of tyrosine. Furthermore, according to an immunological analysis, glycans attached to flagellin via alkaline sensitive linkages may be different from those attached via alkaline resistant linkages.

Bedouet, L., F. Arnold, et al. (1998). "Partial analysis of the flagellar antigenic determinant recognized by a monoclonal antibody to Clostridium tyrobutyricum." Microbiol Immunol **42**(2): 87-95.

In order to count Clostridium tyrobutyricum spores in milk after membrane filtration, murine 21E7-B12 monoclonal antibody was produced. Elution of the monoclonal antibody from this antigen, the flagellar filament protein, by carbohydrate ligands was used to study the epitope structure. A competitive elution of an anti-dextran monoclonal antibody by carbohydrate ligands served as a control in order to validate the immunological tool applied to flagellin epitope study. The carbohydrate moiety of flagellin contained D-glucose and N-acetyl-glucosamine in a molar ration of 11:1 as determined by gas-liquid chromatography and 2 low-abundancy unidentified compounds. In ELISA, D-glucose and N-acetyl-glucosamine did not dissociate the antibody-flagellin complex contrary to maltose, maltotriose, maltotetraose and maltopentaose. The efficiency of elution increased from the dimer to the pentamer and became nil for maltohexaose and maltoheptaose. The fact that the hexamer and heptamer could not react with the 21E7-B12 monoclonal antibody could be explained by a drastic conformational change. The over-all stretched maltopentaose switch to a helical-shaped maltoheptaose which could not fit the 21E7-B12 monoclonal antibody antigen-combining site. Thus, flagellin epitope may contain alpha (1-->4) linked glucose residues plus either N-actyl-glucosamine or an unidentified compound that maintain it in an extended shape.

Beguin, P. and H. Eisen (1978). "Purification and partial characterization of three extracellular cellulases from Cellulomonas sp." Eur J Biochem **87**(3): 525-31.

Three extracellular cellulases have been purified from cultures of Cellulomonas. One was found in solution in the cell-free supernatant and two others were found to be bound to the cellulose added as a carbon source. The free enzyme and one of the cellulose-bound enzymes bind to Sephadex. The two cellulose-bound enzymes are glycosylated. The three enzymes behave as endocellulases towards soluble carboxymethyl-cellulose and have little activity on cellulose powder.

Beguin, P. and M. Lemaire (1996). "The cellulosome: an exocellular, multiprotein complex specialized in cellulose degradation." Crit Rev Biochem Mol Biol **31**(3): 201-36.

Clostridium thermocellum produces a highly active cellulase system that consists of a high-M(r) multienzyme complex termed cellulosome. Hydrolytic components

of the cellulosome are organized around a large, noncatalytic glycoprotein termed CipA that acts both as a scaffolding component and a cellulose-binding factor. Catalytic subunits of the cellulosome bear conserved, noncatalytic subdomains, termed dockerin domains, which bind to receptor domains of CipA, termed cohesin domains. CipA includes nine cohesin domains, a cellulose-binding domain, and a specialized dockerin domain. Proteins of the cell envelope carrying cohesin domains that specifically bind the dockerin domain of CipA have been identified. These proteins may mediate anchoring of the cellulosomes to the cell surface. Cellulase complexes similar to the cellulosome of C. thermocellum are produced by several cellulolytic clostridia. High-M(r) multienzyme complexes have also been identified in anaerobic rumen fungi. The architecture of the fungal complexes also seems to rely on the interaction of conserved, noncatalytic docking domains with a scaffolding component. However, the sequence of the fungal docking domains bears no resemblance to the clostridial dockerin domains, suggesting that the fungal and clostridial complexes arose independently.

Bensing, B. A., B. W. Gibson, et al. (2004). "The Streptococcus gordonii platelet binding protein GspB undergoes glycosylation independently of export." J Bacteriol **186**(3): 638-45.

The binding of bacteria and platelets may play a central role in the pathogenesis of infective endocarditis. Platelet binding by Streptococcus gordonii strain M99 is predominantly mediated by the 286-kDa cell wall-anchored protein GspB. This unusually large protein lacks a typical amino-terminal signal peptide and is translocated from the cytoplasm via a dedicated transport system. A 14-kb segment just downstream of gspB encodes SecA2 and SecY2, two components of the GspB-specific transport system. The downstream segment also encodes several putative glycosyl transferases that may be responsible for the posttranslational modification of GspB. In this study, we compared the abilities of M99 and two GspB(-) mutant strains to bind various lectins. GspB was found to have affinity for lectins that bind N-acetylglucosamine. We also examined variant forms of GspB that lack a carboxy-terminal cell wall-anchoring domain and thus are free of covalent linkage to cell wall peptidoglycan. Like native GspB, these truncated proteins appear to be heavily glycosylated, as evidenced by migration during sodium dodecyl sulfate-polyacrylamide gel electrophoresis with an apparent molecular mass >100 kDa in excess of the predicted mass, negligible staining with conventional protein stains, and reactivity with hydrazide following periodate oxidation. Furthermore, analysis of the carbohydrate associated with the GspB variants by high-pH anion-exchange chromatography revealed the presence of approximately 70 to 100 monosaccharide residues per GspB polypeptide (primarily N-acetylglucosamine and glucose). Analysis of GspB in protoplasts of secA2 or secY2 mutant strains, which do not export GspB, indicates that GspB is glycosylated in the cytoplasm of these strains. The combined data suggest that the native GspB is a glycoprotein and that it may be glycosylated prior to export.

Benz, I. and M. A. Schmidt (2001). "Glycosylation with heptose residues mediated by the aah gene product is essential for adherence of the AIDA-I adhesin." Mol Microbiol **40**(6): 1403-13.

The diffuse adherence of Escherichia coli strain 2787 (O126:H27) is mediated by the autotransporter adhesin AIDA-I (adhesin-involved-in-diffuse-adherence) encoded by the plasmid-borne aidA gene. AIDA-I exhibits an aberrant mobility in denaturing gel electrophoresis. Deletion of the open reading frame (ORF) A immediately upstream of aidA restores the predicted mobility of AIDA-I, but the adhesin is no longer functional. This indicates that the mature AIDA-I adhesin is post-translationally modified and the modification is essential for adherence function. Labelling with digoxigenin hydrazide shows AIDA-I to be glycosylated. Using carbohydrate composition analysis, AIDA-I contains exclusively heptose residues (ratio heptose:AIDA-I approximately 19:1). The deduced amino acid sequence of the cytoplasmic open reading frame (ORF) A gene product shows homologies to heptosyltransferases. In addition, the modification was completely abolished in an ADP-glycero-manno-heptopyranose mutant. Our results provide direct evidence for glycosylation of the AIDA-I adhesin by heptoses with the ORF A gene product as a specific (mono)heptosyltransferase generating the functional mature AIDA-I adhesin. Consequently, the ORF A gene has been denoted 'aah' (autotransporter-adhesin-heptosyltransferase). Glycosylation by heptoses represents a novel protein modification in eubacteria.

Bock, K., J. Schuster-Kolbe, et al. (1994). "Primary structure of the O-glycosidically linked glycan chain of the crystalline surface layer glycoprotein of Thermoanaerobacter thermohydrosulfuricus L111-69. Galactosyl tyrosine as a novel linkage unit." J Biol Chem **269**(10): 7137-44.

The products of Pronase digestion of the crystalline surface layer (S-layer) glycoproteins of Thermoanaerobacter thermohydrosulfuricus strains L111-69 and L110-69 were isolated by gel permeation chromatography, cation exchange chromatography, chromatofocusing, and reversed phase high performance liquid chromatography. Four compounds were obtained which were analyzed by monosaccharide analysis, one- and two-dimensional 500 and 600 MHz 1H and 13C NMR spectroscopy, methylation analysis, gas-liquid chromatography/mass spectrometry, and matrix-assisted laser desorption ionization mass spectrometry. For all glycopeptides we propose the following glycan structure with galactose as the linkage sugar. [formula: see text] The isolated glycopeptides resulted from Pronase cleavage at the glycosylated tyrosine residues. Tyrosine was found as the linkage amino acid in all fractions but the remaining amino acid sequences varied, indicating the presence of different glycosylation sites in the intact S-layer glycoprotein.

Bozal, N. and J. Guinea (1997). "Assembly Properties of a Glycoprotein Produced by Pseudoalteromonas antarctica, NF3." J Colloid Interface Sci **192**(2): 286-93.

The self-assembly properties of an extracellular material of glycoprotein character produced by a new Gram-negative species, NF3, Pseudoalteromonas antarctica, isolated from muddy soil samples of Antarctica have been

investigated. The aggregation behavior of this exopolymer was studied directly by transmission electron microscopy (TEM) and analysis of digitalized TEM images of its aqueous dispersions before and after sonication. Increasing amounts of glycoprotein (GP) in water led to an abrupt decrease in the dispersion surface tensions up to a GP concentration of about 0.20 mg/ml (from 72 to 47 mN m-1), followed by an almost constant surface tension value. The size distribution curves of the aggregates formed always showed a bimodal distribution. The mean size of these two aggregates increased as GP concentration increased (first peak from 120 to 140 nm and second peak from 500 to 700 nm), reaching in both cases almost a constant value also for 0.20 mg of GP/ml of water. TEM images of unsonicated aqueous GP dispersions at concentrations lower and higher than 0.20 mg/ml always showed the coexistence of concentric multilamellar and small unilamellar aggregates, the small particles being the dominant class in the first case. Sonication of these dispersions revealed that each lamella of the initial multilamellar structures was made up of various subunits of coiled coil, whereas the smaller particles were not composed of these subunits. Profiles from digitalized TEM images of unsonicated and sonicated dispersions confirm that each lamella of large aggregates was composed of three subunits. Copyright 1997Academic Press

Brahamsha, B. and E. P. Greenberg (1988). "Biochemical and cytological analysis of the complex periplasmic flagella from Spirochaeta aurantia." J Bacteriol **170**(9): 4023-32.

The periplasmic flagella of Spirochaeta aurantia were isolated and were found to be ultrastructurally and biochemically complex. Generally, flagellar filaments were 18 to 20 nm in diameter and appeared to consist of an 11 to 13-nm-wide inner region and an outer layer. The hook-basal body region consisted of two closely apposed disks connected to a hook by a rod. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified flagella together with a Western blot analysis of a motility mutant that produces hooks and basal bodies but not flagellar filaments revealed that the filaments were composed of three major polypeptides of 37,500, 34,000, and 31,500 apparent molecular weight (37.5K, 34K, and 31.5K polypeptides) and three minor polypeptides of 36,000, 33,000, and 32,000 apparent molecular weight (36K, 33K, and 32K polypeptides). Purified hook-basal body preparations were greatly enriched in three polypeptides in the range of 62,000 to 66,000 apparent molecular weight. Immunogold labeling experiments with a monoclonal antibody specific for the 37.5K flagellin and one that reacts with an epitope common to the 36K, 34K, 33K, 32K, and 31.5K flagellins revealed that the 37.5K major polypeptide was a component of the outer layer, whereas one or more of the other polypeptides constituted the core.

Brechtel, E., M. Matuschek, et al. (1999). "Cell wall of Thermoanaerobacterium thermosulfurigenes EM1: isolation of its components and attachment of the xylanase XynA." Arch Microbiol **171**(3): 159-65.

Thermoanaerobacterium thermosulfurigenes EM1 has a gram-positive type cell

wall completely covered by a surface layer (S-layer) with hexagonal lattice symmetry. The components of the cell envelope were isolated, and the S-layer protein was purified and characterized. S-layer monomers assembled in vitro into sheets with the same hexagonal symmetry as in vivo. Monosaccharide analysis revealed that the S-layer is associated with fucose, rhamnose, mannosamine, glucosamine, galactose, and glucose. The N-terminal 31 amino acid residues of the S-layer protein showed significant similarity to SLH (S-layer homology) domains found in S-layer proteins of different bacteria and in the exocellular enzymes pullulanase, polygalacturonate hydrolase, and xylanase of T. thermosulfurigenes EM1. The xylanase from T. thermosulfurigenes EM1 was copurified with the S-layer protein during isolation of cell wall components. Since SLH domains of some structural proteins have been shown to anchor these proteins noncovalently to the cell envelope, we propose a common anchoring mechanism for the S-layer protein and exocellular enzymes via their SLH domains in the peptidoglycan-containing layer of T. thermosulfurigenes EM1.

Brimer, C. D. and T. C. Montie (1998). "Cloning and comparison of fliC genes and identification of glycosylation in the flagellin of Pseudomonas aeruginosa a-type strains." J Bacteriol **180**(12): 3209-17.

Pseudomonas aeruginosa a-type strains produce flagellin proteins which vary in molecular weight between strains. To compare the properties of a-type flagellins, the flagellin genes of several Pseudomonas aeruginosa a-type strains, as determined by interaction with specific anti-a monoclonal antibody, were cloned and sequenced. PCR amplification of the a-type flagellin gene fragments from five strains each yielded a 1.02-kb product, indicating that the gene size is not likely to be responsible for the observed molecular weight differences among the a-type strains. The flagellin amino acid sequences of several a-type strains (170,018, 5933, 5939, and PAK) were compared, and that of 170,018 was compared with that of PAO1, a b-type strain. The former comparisons revealed that a-type strains are similar in amino acid sequence, while the latter comparison revealed differences between 170,018 and PAO1. Posttranslational modification was explored for its contribution to the observed differences in molecular weight among the a-type strains. A biotin-hydrazide glycosylation assay was performed on the flagellins of three a-type strains (170,018, 5933, and 5939) and one b-type strain (M2), revealing a positive glycosylation reaction for strains 5933 and 5939 and a negative reaction for 170,018 and M2. Deglycosylation of the flagellin proteins with trifluoromethanesulfonic acid (TFMS) confirmed the glycosylation results. A molecular weight shift was observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis for the TFMS-treated flagellins of 5933 and 5939. These results indicate that the molecular weight discrepancies observed for the a-type flagellins can be attributed, at least in part, to glycosylation of the protein. Anti-a flagellin monoclonal antibody reacted with the TFMS-treated flagellins, suggesting that the glycosyl groups are not a necessary component of the epitope for the human anti-a monoclonal antibody. Comparisons between a-type sequences and a b-type sequence (PAO1) will aid in delineation of the epitope for this monoclonal

antibody.

Brockl, G., M. Behr, et al. (1991). "Analysis and nucleotide sequence of the genes encoding the surface-layer glycoproteins of the hyperthermophilic methanogens Methanothermus fervidus and Methanothermus sociabilis." Eur J Biochem **199**(1): 147-52.

The genes (slgA) encoding the surface-layer glycoproteins of the hyperthermophilic methanogens Methano-thermus pervidus and Methanothermus sociabilis were cloned and sequenced. The nucleotide sequences of these genes differ at only nine positions, resulting in three amino acid differences. In both organisms, the transcription start site was localized by primer extension analyses. The DNA sequence at this site conforms to the promotor box B motif for promotors of archaea. 24 nucleotides upstream of the transcription start is an A + T-rich region, which closely resembles the consensus box A motif of promoters of methanogens. Ribosome binding sites are exactly complementary to the 3' end of the 16S rRNA of these methanogens. Both slgA genes encode for a precursor of the mature surface-layer protein containing 593 amino acid residues with a putative N-terminal signal sequence of 22 amino acid residues. The deduced protein sequences contain 20 sequon structures representing possible carbohydrate-binding sites. In comparison with other surface-layer proteins, these obtained from the two hyperthermophilic methanogens contain unusually high amounts of isoleucine, asparagine and cysteine residues. Predicted secondary structures have a high content of beta-sheet structure (44%) and only 7% alpha-helix structures.

Burchard, R. P. and M. L. Sorongon (1998). "A gliding bacterium strain inhibits adhesion and motility of another gliding bacterium strain in a marine biofilm." Appl Environ Microbiol **64**(10): 4079-83.

Two species of gliding bacteria were isolated from a marine biofilm. They were described and identified as members of the genus Cytophaga. One of them (RB1057) produced an extracellular inhibitor of colony expansion of the other (RB1058). The inhibitor was characterized as a glycoprotein with an apparent molecular mass of 60 kDa. It inhibited RB1058 adhesion to and gliding on substrata. Motility and adhesion of several other aquatic gliding bacteria were not measurably affected by this agent.

Calabi, E., S. Ward, et al. (2001). "Molecular characterization of the surface layer proteins from Clostridium difficile." Mol Microbiol **40**(5): 1187-99.

Many bacteria express a surface-exposed proteinaceous layer, termed the S-layer, which forms a regular two-dimensional array visible by electron microscopy. Clostridium difficile is unusual in expressing two S-layer proteins (SLPs), which are of varying size in a number of strains. In an approach combining molecular biology with mass spectrometric sequencing strategies, we have identified the structural gene (slpA) for the S-layer from three strains of C. difficile. Both proteins are derived from a common precursor, and processing involves the removal of a signal peptide and a second cleavage to release the

two mature SLPs. To our knowledge, this is the first example in which two SLPs have been shown to derive from a single gene product through post-translational processing, rather than from the expression of separate genes. The higher molecular weight (MW) SLP is highly conserved among the three strains, whereas the lower MW SLP shows considerable sequence diversity, reflecting the results from Western blotting. The high-MW SLP shows weak homology to N-acetyl muramoyl-L-alanine amidase from Bacillus subtilis, and both the native SLP from C. difficile and a recombinant protein expressed in Escherichia coli were found to display amidase activity by zymography. The high-MW SLPs showed evidence of glycosylation, whereas the lower MW proteins did not. A family of genes with sequence homology to the amidase domain of the high-MW SLP was identified in the C. difficile strain 630 genome, some of which are located in the same region of the genome as slpA and were shown by reverse transcription-polymerase chain reaction (RT-PCR) analysis to be transcribed.

Cerquetti, M., A. Molinari, et al. (2000). "Characterization of surface layer proteins from different Clostridium difficile clinical isolates." Microb Pathog **28**(6): 363-72.

In a previous study we suggested that two surface proteins of a Clostridium difficile strain were involved in the formation of a regularly assembled surface layer (S-layer) external to the cell wall. In the present paper six C. difficile strains isolated from cases and healthy carriers were studied. By using freeze-etching and negative staining techniques two superimposed structurally different lattices were detected on the cell surface of the different C. difficile strains. In each strain, the outer S-layer lattice was arranged in a square symmetry and the inner S-layer lattice in hexagonal symmetry. The S-layer proteins from the different strains were isolated and characterized. Each strain showed two distinct S-layer glycoproteins ranging in molecular mass 36-56 kDa. Antigenic cross-reactivity among the S-layer proteins of higher molecular masses extracted from each strain was demonstrated whereas no antigenic relationship was observed among the different S-layer proteins of lower molecular masses. N-terminal sequence analysis showed the presence of common structural motifs conserved among the high S-layer proteins as well as among the low S-layer proteins. These data indicate that the presence of S-layer on C. difficile strains is common and that its glycoprotein subunits show a certain degree of heterogeneity.

Chakrabarty, A. K., M. A. Maire, et al. (1982). "SDS-PAGE analysis of M. leprae protein antigens reacting with antibodies from sera from lepromatous patients and infected armadillos." Clin Exp Immunol **49**(3): 523-31.

Studies have been conducted to characterize M. leprae bacilli derived from infected armadillos. First, the proteins of the mycobacterial extracts were fractionated by SDS-PAGE. Subsequently, the proteins in the gel were electrophoretically transferred on a strip of nitrocellulose paper by the technique of 'electrophoretic blotting'. The separated bacterial protein bands, thus immobilized on the nitrocellulose paper were made to react immunologically with sera from the lepromatous patients, infected armadillo sera and other experimental mycobacterial antisera. It was observed that a majority of M. leprae

proteins contained antigenic determinants also present on proteins of BCG. In addition, only two specific antigen bands of 33KD and 12KD were conspicuously detected by the patients' sera and the infected armadillo sera. These substances were further identified as polysaccharides or glycoproteins since they could only be stained by Schiff's reagent or alcian blue. Only 12KD glycoprotein band reacted with concanavalin A, whereas wheat germ agglutinin (WGA) did not show any reaction with them. These 33KD and 12KD glycoprotein antigens were found to lose their antigenicity after pepsin treatment and can be considered as glycoproteins. Further, radiolabelling experiments showed that 12KD antigen underwent radioiodination under usual conditions, but 33KD glycoprotein failed to be similarly radiolabelled. It is suggested that these protein antigens have M. leprae specific determinants on a cross-reacting component.

Charlton, S., A. J. Moir, et al. (1999). "Characterization of the exosporium of Bacillus cereus." J Appl Microbiol **87**(2): 241-5.

Exosporium components from endospores of Bacillus cereus ATCC 10876 were purified and separated by gel electrophoresis. Several of the proteins for which N-terminal sequences were recovered were found to have homologues in protein databases which have been demonstrated to have enzymic activity in other organisms. Amongst these is a zinc metalloprotease, immune inhibitor A, already described in B. thuringiensis. This has been shown to be present in an active 73 kDa form on the exosporium of B. cereus. Other proteins associated with the exosporium include the molecular chaperone GroEL and a homologue of RocA (1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12)) of B. subtilis. Although these are unlikely to represent integral structural proteins of the exosporium, the observation that they are selectively present in the spore surface layer suggests that this layer may have functional significance.

Che, F. S., Y. Nakajima, et al. (2000). "Flagellin from an incompatible strain of Pseudomonas avenae induces a resistance response in cultured rice cells." J Biol Chem **275**(41): 32347-56.

The host range of Pseudomonas avenae is wide among monocotyledonous plants, but individual strains can infect only one or a few host species. The resistance response of rice cells to pathogens has been previously shown to be induced by a rice-incompatible strain, N1141, but not by a rice-compatible strain, H8301. To clarify the molecular mechanism of the host specificity in P. avenae, a strain-specific antibody that was raised against N1141 cells and then absorbed with H8301 cells was prepared. When a cell extract of strain N1141 was separated by SDS-polyacrylamide gel electrophoresis and immunostained with the N1141 strain-specific antibody, only a flagellin protein was detected. Purified N1141 flagellin induced the hypersensitive cell death in cultured rice cells within 6 h of treatment, whereas the H8301 flagellin did not. The hypersensitive cell death could be blocked by pretreatment with anti-N1141 flagellin antibody. Furthermore, a flagellin-deficient N1141 strain lost not only the induction ability of hypersensitive cell death but also the expression ability of the EL2 gene, which is thought to be one of the defense-related genes. These results demonstrated that

the resistance response in cultured rice cells is induced by the flagellin existing in the incompatible strain of P. avenae but not in the flagellin of the compatible strain.

Chen, J. R., J. H. Lin, et al. (1998). "Identification of a novel adhesin-like glycoprotein from Mycoplasma hyopneumoniae." Vet Microbiol **62**(2): 97-110.

This study identifies an adhesin-like glycoprotein, which was a 110 kDa protein (P110) under HPLC-GPC assay. This adhesin consisted of one P54 and two P28 subunits. In addition, N-glycosidase F could cleave all N-linked oligosaccharides on the P54 subunit. Experimental results indicated that P110 with native conformations significantly inhibited the adherence of biotin-labeled porcine tracheal epithelial cell extracts to the intact M. hyopneumoniae cells ($p < 0.01$). Furthermore, the biotin-labeled porcine tracheal epithelial cell extracts specifically bound to P54 and P28 subunits. This binding could be competitively inhibited by unlabeled porcine tracheal epithelial extracts and SPF porcine antisera against Mycoplasma hyopneumoniae. Both P54 and P28 subunits were constitutively expressed in different strains of M. hyopneumoniae. Their production was negligibly changed at various passages during in vitro cultivation. The significant role of this adhesin-like glycoprotein in the pathogenesis of swine pneumonia is under study.

Christian, R., G. Schulz, et al. (1993). "Complete structure of the tyrosine-linked saccharide moiety from the surface layer glycoprotein of Clostridium thermohydrosulfuricum S102-70." J Bacteriol **175**(5): 1250-6.

In this study, we have extended and completed a previous investigation (P. Messner, R. Christian, J. Kolbe, G. Schulz, and U. B. Sleytr, J. Bacteriol. 174:2236-2240, 1992) in which we demonstrated for the first time in prokaryotic organisms the presence of a novel O-glycosidic linkage via tyrosine. The surface layer glycoprotein of the eubacterium Clostridium thermohydrosulfuricum S102-70 is arranged in a hexagonal lattice, with center-to-center spacings of approximately 16.3 nm. Molecular weight determination by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of both glycosylated and chemically deglycosylated surface layer glycoprotein showed values for the monomeric subunits of 94,000 and 87,500, respectively. Glycopeptide fractions obtained after exhaustive pronase digestion of purified, intact glycoprotein were isolated by reversed-phase liquid chromatography. One- and two-dimensional nuclear magnetic resonance studies, together with chemical analyses and plasma desorption time-of-flight mass spectrometry, were used to elucidate the structure of the hexasaccharide moiety linked by the novel O-glycosidic linkage to tyrosine. The combined evidence suggests the following structure: beta-D-Galf-(1-->3)-alpha-D-Galp- (1-->2)-alpha-L-Rhap-(1-->3)-alpha-D-Manp-(1--3)-alpha-L- Rhap-(1-->3)-beta- D-Glcp-(1-->4)-L-Tyr.

Cocera, M., O. Lopez, et al. (2001). "Partitioning of SDS in liposomes coated by the exopolymer excreted by Pseudoalteromonas antarctica NF3 as a measure of vesicle protection against this surfactant." J Biomater Sci Polym Ed **12**(2): 255-66.

The capacity of glycoprotein (GP) excreted by Pseudoalteromonas antarctica NF3, to protect phosphatidylcholine (PC) liposomes against the action of the anionic surfactant sodium dodecyl sulfate (SDS) was studied in detail. To this end, changes in the surfactant partitioning between the lipid bilayer and the aqueous phase (partition coefficients, K) and in the effective surfactant to PC molar ratios (Re) were determined as a function of the amount of GP assembled with liposomes. The permeability of liposomes was determined by monitoring the changes in the fluorescence intensity of liposomes due to the release of the fluorescent dye 5(6)-carboxyfluorescein (CF) from the interior of vesicles to the bulk aqueous phase. Increasing GP amounts in the system resulted in the same interaction step as a rise in Re and a fall in the surfactant partitioning between the lipid bilayer and water. Hence, the higher the proportion of GP, the lower the surfactant ability to alter the permeability of liposomes and the lower its affinity with these bilayer structures. In addition, increasing GP proportions resulted in the same interaction step as a progressive increase of the free surfactant concentration (S(W)). The fact that the S(W) was always lower than the surfactant critical micelle concentration indicates that the interaction of SDS with coated liposomes was mainly ruled by the action of surfactant monomers in all cases.

Cowlishaw, D. A. and M. C. Smith (2001). "Glycosylation of a Streptomyces coelicolor A3(2) cell envelope protein is required for infection by bacteriophage phi C31." Mol Microbiol **41**(3): 601-10.

Mutants of Streptomyces coelicolor A3(2) J1929 (Delta pglY) were isolated that were resistant to the Streptomyces temperate phage phi C31. These strains could be transfected with phi C31 DNA, but could not act as infective centres after exposure to phage. Thus, it was concluded that infection was blocked at the adsorption/DNA injection step. The mutants fell into three classes. Class I mutants were complemented by a gene, SCE87.05, isolated from the cosmid library of S. coelicolor A3(2). The product of SCE87.05 had good overall homology to a Mycobacterium tuberculosis hypothetical protein and regions with similarity to dolichol phosphate-D-mannose:protein O-D-mannosyltransferases. Concanavalin A (ConA) inhibited phi C31 infection of S. coelicolor J1929, and this could be partially reversed by the addition of the sugar, alpha-D-methyl-pyranoside. Moreover, glycosylated proteins from J1929, but not from the class I mutant DT1017, were detected using ConA as a probe in Western blots. Class I and II mutants were sensitive to phi C31hc, a previously isolated phage exhibiting an extended host range phenotype, conferred by h. A phage with the same phenotype, phi DT4002, was isolated independently, and a missense mutation was found in a putative tail gene. It is proposed that the phi C31 receptor is a cell wall glycoprotein, and that the phi C31h mutation compensates for the lack of glycosylation of the receptor.

Cowlishaw, D. A. and M. C. Smith (2002). "A gene encoding a homologue of dolichol phosphate-beta-D-mannose synthase is required for infection of Streptomyces coelicolor A3(2) by phage (phi)C31." J Bacteriol **184**(21): 6081-3.

We have shown previously that a gene encoding a homologue to the eukaryotic dolichol-phosphate-D-mannose, protein O-D-mannosyltransferase, was required for (phi)C31 infection of Streptomyces coelicolor. Here we show that a gene encoding the homologue to dolichol-phosphate-mannose synthase is also essential for phage sensitivity. These data confirm the role of glycosylation in the phage receptor for (phi)C31 in S. coelicolor.

Cuezzo de Gines, S., M. C. Maldonado, et al. (2000). "Purification and characterization of invertase from Lactobacillus reuteri CRL 1100." Curr Microbiol **40**(3): 181-4.

The invertase of Lactobacillus reuteri CRL 1100 is a glycoprotein composed by a single subunit with a molecular weight of 58 kDa. The enzyme was stable below 45 degrees C over a wide pH range (4.5-7.0) with maximum activity at pH 6.0 and 37 degrees C. The invertase activity was significantly inhibited by bivalent metal ions (Ca(++), Cu(++), Cd(++), and Hg(++)), beta-mercaptoethanol, and dithiothreitol and partially improved by ethylenediaminetetraacetic acid. The enzyme was purified 32 times over the crude extract by gel filtration and ion-exchange chromatography with a recovery of 17%. The K(m) and V(max) values for sucrose were 6.66 mM and 0.028 micromol/min, respectively. An invertase is purified and characterized for the first time in Lactobacillus, and it proved to be a beta-fructofuranosidase.

Curtis, M. A., A. Thickett, et al. (1999). "Variable carbohydrate modifications to the catalytic chains of the RgpA and RgpB proteases of Porphyromonas gingivalis W50." Infect Immun **67**(8): 3816-23.

Proteases of Porphyromonas gingivalis are considered to be important virulence determinants of this periodontal bacterium. Several biochemical isoforms of arginine-specific proteases are derived from rgpA and rgpB. HRgpA is a heterodimer composed of the catalytic alpha chain noncovalently associated with a beta adhesin chain derived from the C terminus of the initial full-length translation product. The catalytic alpha chain is also present as a monomer (RgpA) either free in solution or associated with membranes. rgpB lacks the coding region for the adhesin domain present in rgpA and yields only monomeric forms (RgpB) which again may be soluble or membrane associated. In this study, the catalytic chains of this unusual group of enzymes are shown to be differentially modified by the posttranslational addition of carbohydrate. A monoclonal antibody (MAb 1B5) raised to the monomeric RgpA did not react with the corresponding recombinant RgpA alpha chain expressed in Escherichia coli but was immunoreactive with P. gingivalis lipopolysaccharide. MAb 1B5 also reacted with the membrane-associated forms of RgpA and RgpB but not with the heterodimeric HRgpA and the soluble form of RgpB. RgpA treated with denaturants was capable of binding to MAb 1B5 whereas treatment with periodate abolished this binding, suggesting the presence of carbohydrate residues within the epitope. Chemical deglycosylation abolished immunoreactivity with MAb 1B5 and caused a approximately 30% reduction in the size of the membrane-associated enzymes. Monosaccharide analysis of HRgpA and RgpA demonstrated 2.1 and 14.4%, respectively, carbohydrate by

weight of protein. Furthermore, distinct differences were detected in their monosaccharide compositions, indicating that these protease isoforms are modified not only to different extents but also with different sugars. The variable nature of these additions may have a significant effect on the structure, stability, and immune recognition of these protease glycoproteins.

Daubenspeck, J. M., H. Zeng, et al. (2004). "Novel oligosaccharide side chains of the collagen-like region of BclA, the major glycoprotein of the Bacillus anthracis exosporium." J Biol Chem **279**(30): 30945-53.

Spores of Bacillus anthracis, the causative agent of anthrax, are enclosed by a prominent loose fitting layer called the exosporium. The exosporium consists of a basal layer and an external hairlike nap. The filaments of the nap are composed of a highly immunogenic glycoprotein called BclA, which has a long, central collagen-like region with multiple XXG repeats. Most of the triplet repeats are PTG, and nearly all of the triplet repeats contain a threonine residue, providing multiple potential sites for O-glycosylation. In this study, we demonstrated that two O-linked oligosaccharides, a 715-Da tetrasaccharide and a 324-Da disaccharide, are released from spore- and exosporium-associated BclA by hydrazinolysis. Each oligosaccharide is probably attached to BclA through a GalNAc linker, which was lost during oligosaccharide release. We found that multiple copies of the tetrasaccharide are linked to the collagen-like region of BclA, whereas the disaccharide may be attached outside of this region. Using NMR, mass spectrometry, and other analytical techniques, we determined that the structure of the tetrasaccharide is 2-O-methyl-4-(3-hydroxy-3-methylbutamido)-4,6-dideoxy-beta-d-glucopyranosy l-(1-->3)-alpha-l-rhamnopyranosyl-(1-->3)-alpha-l-rhamnopyranosyl-(1-->2)- l-rhamnopyranose. The previously undescribed nonreducing terminal sugar (i.e. 2-O-methyl-4-(3-hydroxy-3-methylbutamido)-4,6-dideoxy-d-glucose) was given the trivial name anthrose. Anthrose was not found in spores of either Bacillus cereus or Bacillus thuringiensis, two species that are the most phylogenetically similar to B. anthracis. Thus, anthrose may be useful for species-specific detection of B. anthracis spores or as a new target for therapeutic intervention.

Degnan, B. A., M. C. Fontaine, et al. (2000). "Characterization of an isogenic mutant of Streptococcus pyogenes Manfredo lacking the ability to make streptococcal acid glycoprotein." Infect Immun **68**(5): 2441-8.

An isogenic mutant of Streptococcus pyogenes Manfredo that lacks the ability to make streptococcal acid glycoprotein (SAGP) has been constructed by inserting a deletion in the sagp gene using the method of allelic exchange. An assay of cell extracts (CE) prepared from the wild-type and mutant Manfredo strains for the enzyme arginine deiminase (AD) showed that significant activity was present in wild-type CE but none could be detected in mutant CE. These findings confirm our earlier conclusion that SAGP has AD activity (B. A. Degnan, J. M. Palmer, T. Robson, C. E. D. Jones, M. Fischer, M. Glanville, G. D. Mellor, A. G. Diamond, M. A. Kehoe, and J. A. Goodacre, Infect. Immun. 66:3050-3058, 1998). Wild-type CE but not mutant CE potently inhibited human peripheral blood mononuclear

cell proliferation in response to phytohemagglutinin, and this inhibition was overcome by the addition of L-arginine to proliferation assay mixtures. Invasion assays showed that the isogenic mutant organisms lacking SAGP, and thus AD activity, were between three and five times less able to enter epithelial cells (Hep-2C and A549) than were the wild-type streptococci. Both wild-type and mutant S. pyogenes bacteria were extremely sensitive to low pH. However, L-arginine (1 mM or above) significantly increased the viability of the wild type but not the isogenic mutant organisms under acidic conditions. The difference in acid susceptibility between wild-type and mutant bacteria may explain the reduced capacity of the isogenic mutant bacteria to invade and survive intracellularly.

Degnan, B. A., J. M. Palmer, et al. (1998). "Inhibition of human peripheral blood mononuclear cell proliferation by Streptococcus pyogenes cell extract is associated with arginine deiminase activity." Infect Immun **66**(7): 3050-8.

Streptococcus pyogenes (group A Streptococcus) cell extracts (CE) have a remarkably powerful and dose-dependent inhibitory effect on antigen, superantigen, or mitogen-stimulated human peripheral blood mononuclear cell (PBMC) proliferation in vitro. Purification of the inhibitory component present in S. pyogenes type M5 (Manfredo strain) CE by anion-exchange chromatography followed by gel filtration chromatography showed that the inhibitor had an approximate native molecular mass of 100 kDa. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified inhibitory fractions followed by silver staining gave a single band with an approximate molecular mass of 47 kDa, indicating that the inhibitor is composed of two identical subunits. NH2-terminal sequencing of the protein revealed that it was identical to the previously characterized streptococcal acid glycoprotein (SAGP); this protein possesses between 31.5 and 39.0% amino acid identity with arginine deiminase (AD) from Mycoplasma hominis, Mycoplasma arginini, Pseudomonas putida, and Pseudomonas aeruginosa. AD enzyme activity was present in unfractionated CE prepared from a range of streptococcal strains, and partially purified inhibitory fractions of Manfredo CE also had high levels of activity. The inhibitory effect of Manfredo CE was overcome by the addition of L-arginine to proliferation assays in which human PBMC were stimulated with phytohemagglutinin. We conclude that SAGP, or its homolog, possesses AD activity and that the potent inhibition of proliferation of human T cells by streptococcal CE is due to activity of this enzyme.

Dobson, W. J. and H. D. McCurdy (1979). "The function of fimbriae in Myxococcus xanthus. I. Purification and properties of M. xanthus fimbriae." Can J Microbiol **25**(10): 1152-60.

Myxococcus xanthus fimbriae have been purified and characterized as part of a study of the function of fimbriae in this prokaryote. Myxococcus xanthus produced two types of fimbriae, termed flaccid (F) and rigid (R) on the basis of electron microscopy. F and R fimbriae differed slightly in their response to pH and freeze-thaw regimes but were similar in their resistance to hydrolytic enzymes, amino acid composition, molecular weight, carbohydrate content, and

antigenic determinants. Although the precise relationship between F and R fimbriae is unknown, the possibility is considered that F fimbriae might represent a "contracted" form of the R type. Studies designed to determine fimbriae function in M. xanthus are described in an accompanying report.

Doig, P., N. Kinsella, et al. (1996). "Characterization of a post-translational modification of Campylobacter flagellin: identification of a sero-specific glycosyl moiety." Mol Microbiol **19**(2): 379-87.

The flagellins of Campylobacter spp. differ antigenically. In variants of C. coli strain VC167, two antigenic flagellin types determined by sero-specific antibodies have been described (termed T1 and T2). Post-translational modification has been suggested to be responsible for T1 and T2 epitopes, and, using mild periodate treatment and biotin hydrazide labelling, flagellin from both VC167-T1 and T2 were shown to be glycosylated. Glycosylation was also shown to be present on other Campylobacter flagellins. The ability to label all Campylobacter flagellins examined with the lectin LFA demonstrated the presence of a terminal sialic acid moiety. Furthermore, mild periodate treatment of the flagellins of VC167 eliminated reactivity with T1 and T2 specific antibodies LAH1 and LAH2, respectively, and LFA could also compete with LAH1 and LAH2 antibodies for binding to their respective flagellins. These data implicate terminal sialic acid as part of the LAH strain-specific epitopes. However, using mutants in genes affecting LAH serorecognition of flagellin it was demonstrated that sialic acid alone is not the LAH epitope. Rather, the epitope(s) is complex, probably involving multiple glycosyl and/or amino acid residues.

Dong, S., O. N. Chesnokova, et al. (2009). "Identification of the UDP-N-acetylglucosamine 4-epimerase involved in exosporium protein glycosylation in Bacillus anthracis." J Bacteriol **191**(22): 7094-101.

Spores of Bacillus anthracis, the causative agent of anthrax, are enclosed by a loosely fitting exosporium composed of a basal layer and an external hair-like nap. The filaments of the nap are formed by trimers of the collagen-like glycoprotein BclA. The side chains of BclA include multiple copies of two linear rhamnose-containing oligosaccharides, a trisaccharide and a pentasaccharide. The pentasaccharide terminates with the unusual deoxyamino sugar anthrose. Both oligosaccharide side chains are linked to the BclA protein backbone through an N-acetylgalactosamine (GalNAc) residue. To identify the gene encoding the epimerase required to produce GalNAc for BclA oligosaccharide biosynthesis, three annotated UDP-glucose 4-epimerase genes of B. anthracis were cloned and expressed in Escherichia coli. The candidate proteins were purified, and their enzymatic activities were assessed. Only two proteins, encoded by the BAS5114 and BAS5304 genes (B. anthracis Sterne designations), exhibited epimerase activity. Both proteins were able to convert UDP-glucose (Glc) to UDP-Gal, but only the BAS5304-encoded protein could convert UDP-GlcNAc to UDP-GalNAc, indicating that BAS5304 was the gene sought. Surprisingly, spores produced by a mutant strain lacking the BAS5304-encoded enzyme still contained normal levels of BclA-attached oligosaccharides. However,

monosaccharide analysis of the oligosaccharides revealed that GlcNAc had replaced GalNAc. Thus, while GalNAc appears to be the preferred amino sugar for the linkage of oligosaccharides to the BclA protein backbone, in its absence, GlcNAc can serve as a substitute linker. Finally, we demonstrated that the expression of the BAS5304 gene occurred in a biphasic manner during both the early and late stages of sporulation.

Easton, D. M., M. Totsika, et al. "Characterization of EhaJ, a New Autotransporter Protein from Enterohemorrhagic and Enteropathogenic Escherichia coli." Front Microbiol **2**: 120.

Enterohemorrhagic Escherichia coli (EHEC) and enteropathogenic E. coli (EPEC) are diarrheagenic pathotypes of E. coli that cause gastrointestinal disease with the potential for life-threatening sequelae. While certain EHEC and EPEC virulence mechanisms have been extensively studied, the factors that mediate host colonization remain to be properly defined. Previously, we identified four genes (ehaA, ehaB, ehaC, and ehaD) from the prototypic EHEC strain EDL933 that encode for proteins that belong to the autotransporter (AT) family. Here we have examined the prevalence of these genes, as well as several other AT-encoding genes, in a collection of EHEC and EPEC strains. We show that the complement of AT-encoding genes in EHEC and EPEC strains is variable, with some AT-encoding genes being highly prevalent. One previously uncharacterized AT-encoding gene, which we have termed ehaJ, was identified in 12/44 (27%) of EHEC and 2/20 (10%) of EPEC strains. The ehaJ gene lies immediately adjacent to a gene encoding a putative glycosyltransferase (referred to as egtA). Western blot analysis using an EhaJ-specific antibody indicated that EhaJ is glycosylated by EgtA. Expression of EhaJ in a recombinant E. coli strain, revealed EhaJ is located at the cell surface and in the presence of the egtA glycosyltransferase gene mediates strong biofilm formation in microtiter plate and flow cell assays. EhaJ also mediated adherence to a range of extracellular matrix proteins, however this occurred independent of glycosylation. We also demonstrate that EhaJ is expressed in a wild-type EPEC strain following in vitro growth. However, deletion of ehaJ did not significantly alter its adherence or biofilm properties. In summary, EhaJ is a new glycosylated AT protein from EPEC and EHEC. Further studies are required to elucidate the function of EhaJ in colonization and virulence.

Egelseer, E. M., T. Danhorn, et al. (2001). "Characterization of an S-layer glycoprotein produced in the course of S-layer variation of Bacillus stearothermophilus ATCC 12980 and sequencing and cloning of the sbsD gene encoding the protein moiety." Arch Microbiol **177**(1): 70-80.

The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered by an oblique lattice which consists of the S-layer protein SbsC. On SDS-polyacrylamide gels, the mature S-layer protein migrates as a single band with an apparent molecular mass of 122 kDa. During cultivation of B. stearothermophilus ATCC 12980 at 67 degrees C instead of 55 degrees C, a variant developed that had a secondary cell wall polymer identical to that of the

wild-type strain, but it carried an S-layer glycoprotein that could be separated on SDS-polyacrylamide gels into four bands with apparent molecular masses of 92, 118, 150 and 175 kDa. After deglycosylation, only a single protein band with a molecular mass of 92 kDa remained. The complete nucleotide sequence encoding the protein moiety of this S-layer glycoprotein, termed SbsD, was established by PCR and inverse PCR. The sbsD gene of 2,709 bp is predicted to encode a protein of 96.2 kDa with a 30-amino-acid signal peptide. Within the 807 bp encoding the signal peptide and the N-terminal sequence (amino acids 31-269), different nucleotides for sbsD and sbsC were observed in 46 positions, but 70% of these mutations were silent, thus leading to a level of identity of 95% for the N-terminal parts. The level of identity of the remaining parts of SbsD and SbsC was below 10%, indicating that the lysine-, tyrosine- and arginine-rich N-terminal region in combination with a distinct type of secondary cell wall polymer remained conserved upon S-layer variation. The sbsD sequence encoding the mature S-layer protein cloned into the pET28a vector led to stable expression in Escherichia coli HMS174(DE3). This is the first example demonstrating that S-layer variation leads to the synthesis of an S-layer glycoprotein.

Eichler, J. (2000). "Novel glycoproteins of the halophilic archaeon Haloferax volcanii." Arch Microbiol **173**(5-6): 445-8.

Archaea possess many eukaryote-like properties, including the ability to glycosylate proteins. Using oligosaccharide staining and lectin binding, this study revealed the existence of several glycosylated Haloferax volcanii membrane proteins, besides the previously reported surface layer (S-layer) glycoprotein. While the presence of glycoproteins in archaeal S-layers and flagella is well-documented, few archaeal glycoproteins that are not part of these structures have been reported. The glycosylated 150, 98, 58 and 54 kDa protein species detected were neither precursors nor breakdown products of the 190 kDa S-layer glycoprotein. Furthermore, these novel glycoproteins were outwardly oriented and intimately associated with the membrane.

Eichler, J. and M. W. Adams (2005). "Posttranslational protein modification in Archaea." Microbiol Mol Biol Rev **69**(3): 393-425.

One of the first hurdles to be negotiated in the postgenomic era involves the description of the entire protein content of the cell, the proteome. Such efforts are presently complicated by the various posttranslational modifications that proteins can experience, including glycosylation, lipid attachment, phosphorylation, methylation, disulfide bond formation, and proteolytic cleavage. Whereas these and other posttranslational protein modifications have been well characterized in Eucarya and Bacteria, posttranslational modification in Archaea has received far less attention. Although archaeal proteins can undergo posttranslational modifications reminiscent of what their eucaryal and bacterial counterparts experience, examination of archaeal posttranslational modification often reveals aspects not previously observed in the other two domains of life. In some cases, posttranslational modification allows a protein to survive the extreme conditions often encountered by Archaea. The various posttranslational modifications

experienced by archaeal proteins, the molecular steps leading to these modifications, and the role played by posttranslational modification in Archaea form the focus of this review.

Emery, D. L., B. L. Clark, et al. (1984). "Analysis of the outer membrane proteins of Bacteroides nodosus, the causal organism of ovine footrot." Vet Microbiol **9**(2): 155-68.
Examination by SDS-PAGE of lithium acetate extracts of several strains of depiliated Bacteroides nodosus revealed 6 major outer membrane proteins (including pilin). The 5 membrane proteins exhibited approximate molecular weights of 75000, 50000, 38000, 34500 and 26500 whereas pilin had a MW of 17500 for the majority of strains. All proteins were accessible to lactoperoxidase-catalysed iodination and proteins 1, 2 and 5 were shown to be glycoproteins. Several attempts to isolate individual OMC proteins in pure form by selective solubilization and gel filtration were unsuccessful, but electroelution of individual outer membrane complex proteins resolved by SDS-PAGE provided sufficient quantities of antigen for immunization of sheep and for immunochemical analysis.

Erickson, P. R. and M. C. Herzberg (1993). "Evidence for the covalent linkage of carbohydrate polymers to a glycoprotein from Streptococcus sanguis." J Biol Chem **268**(32): 23780-3.
The platelet aggregation-associated protein (PAAP) from Streptococcus sanguis contains 39% carbohydrate in rhamnose-rich polysaccharides. Synthesized by cultured protoplasts, these polysaccharides were shown to be covalently linked to this cell wall protein using specific inhibitors of glycosylation, glycosidase treatment, amino acid and carbohydrate analyses of prepared minimal glycopeptides and isolated protein, and NMR spectroscopy. To our knowledge, this is the first direct proof of an N-asparaginyl linkage of carbohydrate to a eubacterial protein.

Espitia, C., R. Espinosa, et al. (1995). "Antigenic and structural similarities between Mycobacterium tuberculosis 50- to 55-kilodalton and Mycobacterium bovis BCG 45- to 47-kilodalton antigens." Infect Immun **63**(2): 580-4.
The relationship between Mycobacterium tuberculosis 50- to 55-kDa protein and Mycobacterium bovis BCG 45- to 47-kDa antigen was examined by using immunological and biochemical criteria. Reciprocal cross-reactivity with a rabbit polyclonal antiserum against the M. bovis BCG protein and with a monoclonal antibody raised against the M. tuberculosis antigen was observed. The epitope recognized by this antibody was apparently present only in proteins of M. tuberculosis and M. bovis BCG among the 11 mycobacterial species tested. The amino-terminal sequences and total amino acid contents of these proteins showed strong similarities. Both antigens are glycoproteins as assessed by binding of concanavalin A, labeling of carbohydrate moieties with biotin-hydrazide, and digestion of carbohydrates with jack bean alpha-D-mannosidase, which produced a reduction of the molecular weights of the proteins and totally eliminated concanavalin A binding. Both M. tuberculosis and M. bovis BCG

proteins are secreted, since they were found mainly in the culture medium. Analysis of M. tuberculosis 50- to 55-kDa antigen by two-dimensional gel electrophoresis showed at least seven different components, as previously described for the M. bovis BCG antigen. Solid-phase immunoassays showed that the purified M. tuberculosis 50- to 55-kDa protein was recognized by serum specimens from 70% of individuals with pulmonary tuberculosis from a total of 77 Mexican patients examined.

Espitia, C. and R. Mancilla (1989). "Identification, isolation and partial characterization of Mycobacterium tuberculosis glycoprotein antigens." Clin Exp Immunol **77**(3): 378-83.
In Mycobacterium tuberculosis culture filtrates, three concanavalin A (ConA)-binding bands of 55, 50 and 38 kilodaltons (kD) were identified by labelling blotted proteins with a ConA-peroxidase conjugate. Binding was inhibited by the competitor sugar alpha-methyl mannoside and by reduction with sodium m-periodate. Bands of 55, 50 and 38 kD stained with Coomasie blue were sensitive to digestion with proteases, thus indicating that they are proteins. Glycoproteins were isolated by lectin affinity chromatography or by elution from nitrocellulose membranes. On the isolated form, the 55-50 kD doublet glycoprotein was 65.4% protein and 34.6% sugar. The purified 38 kD molecule was 74.3% protein and 25.7% carbohydrate. By immunoblot, antibodies against mycobacterial glycoproteins were demonstrated in immunized rabbits and in patients with pulmonary tuberculosis, but not in healthy individuals. Treatment with sodium m-periodate abolished binding of rabbit antibodies to the 38 kD glycoprotein. Reactivity of the 55-50 kD doublet glycoprotein was not altered by reduction. By immunoblot with monoclonal antibodies TB71 and TB72, a carbohydrate-dependent and a carbohydrate-independent epitope could be identified on the 38 kD glycoprotein.

Faguy, D. M., D. P. Bayley, et al. (1996). "Isolation and characterization of flagella and flagellin proteins from the Thermoacidophilic archaea Thermoplasma volcanium and Sulfolobus shibatae." J Bacteriol **178**(3): 902-5.
Isolated flagellar filaments of Sulfolobus shibatae were 15 nm in diameter, and they were composed of two major flagellins which have M(r)s of 31,000 and 33,000 and which stained positively for glycoprotein. The flagellar filaments of Thermoplasma volcanium were 12 nm in diameter and were composed of one major flagellin which has an M(r) of 41,000 and which also stained positively for glycoprotein. N-terminal amino acid sequencing indicated that 18 of the N-terminal 20 amino acid positions of the 41-kDa flagellin of T. volcanium were identical to those of the Methanococcus voltae 31-kDa flagellin. Both flagellins of S. shibatae had identical amino acid sequences for at least 23 of the N-terminal positions. This sequence was least similar to any of the available archaeal flagellin sequences, consistent with the phylogenetic distance of S. shibatae from the other archaea studied.

Fletcher, C. M., M. J. Coyne, et al. (2007). "Phase-variable expression of a family of glycoproteins imparts a dynamic surface to a symbiont in its human intestinal

ecosystem." Proc Natl Acad Sci U S A **104**(7): 2413-8.

The recent report of the synthesis of glycoproteins by the abundant intestinal symbionts Bacteroides showed that these organisms use a novel bacterial enzyme to decorate their surfaces with a sugar residue derived from their environment. As a first step in understanding the importance of these glycoproteins to the bacteria and to the bacterial-host symbiosis, we identified and characterized the abundant glycoproteins of Bacteroides distasonis (proposed reclassification as Parabacteroides distasonis) [Sakamoto M, Benno Y (2006) Int J Syst Evol Microbiol 56:1599-1605]. Using lectin-affinity purification followed by tandem mass spectrometry, we identified a family of at least nine glycoproteins, similar only to the S-layer glycoproteins of Tannerella forsythia. Analysis of one of these purified glycoproteins demonstrated that the glycan is primarily a polymer of xylose, a monosaccharide rarely found in bacterial glycans. Even more unexpected was the finding that seven of nine of the glycoprotein promoters undergo DNA inversion, a process that we show is active in their endogenous human environment. Using cross-species functional assays, we show that a single serine family site-specific recombinase globally mediates the inversions of these glycoprotein promoters. This regulatory mechanism is similar to that of the Bacteroides fragilis capsular polysaccharides and establishes DNA inversion as a general and ancient means of regulation of glycan-containing surface molecules of these important human intestinal symbionts.

Fletcher, C. M., M. J. Coyne, et al. (2009). "A general O-glycosylation system important to the physiology of a major human intestinal symbiont." Cell **137**(2): 321-31.

The Bacteroides are a numerically dominant genus of the human intestinal microbiota. These organisms harbor a rare bacterial pathway for incorporation of exogenous fucose into capsular polysaccharides and glycoproteins. The infrequency of glycoprotein synthesis by bacteria prompted a more detailed analysis of this process. Here, we demonstrate that Bacteroides fragilis has a general O-glycosylation system. The proteins targeted for glycosylation include those predicted to be involved in protein folding, protein-protein interactions, peptide degradation as well as surface lipoproteins. Protein glycosylation is central to the physiology of B. fragilis and is necessary for the organism to competitively colonize the mammalian intestine. We provide evidence that general O-glycosylation systems are conserved among intestinal Bacteroides species and likely contribute to the predominance of Bacteroides in the human intestine.

Gandolfi-Donadio, L., G. Gola, et al. (2006). "Synthesis of alpha-D-Gal f-(1-->2)-D-galactitol and alpha-D-Gal f-(1-->2)[beta-D-Gal f-(1-->3)]-D-galactitol, oligosaccharide derivatives from Bacteroides cellulosolvens glycoproteins." Carbohydr Res **341**(15): 2487-97.

The synthesis of alpha-D-galactofuranosyl-(1-->2)-D-galactitol, which has been isolated by reductive beta-elimination from glycoproteins of Bacteroides cellulosolvens and Clostridium thermocellum, is described. The approach of

selective glycosylation of an aldono-1,4-lactone by the trichloroacetimidate method was employed. The synthesis of alpha-D-Gal f-(1-->2)[beta-D-Gal f-(1-->3)]-D-Galol, that contains Gal f units in both anomeric configurations, is also reported. These are the first synthetic oligosaccharides with alpha-D-Gal f, previously found in natural products.

Garcia-Patrone, M. and J. S. Tandecarz (1995). "A glycoprotein multimer from Bacillus thuringiensis sporangia: dissociation into subunits and sugar composition." Mol Cell Biochem **145**(1): 29-37.

Two glycoproteins (205 and 72 kDa) were found in Bacillus thuringiensis sporangia. They were predominantly localized in the exosporium and/or the spore coat, although a small proportion was also found in membranes. A method for the dissociation of hydrophobic aggregates that resist the usual conditions of SDS-PAGE is described. Using this method we established that the 205 kDa glycoprotein is a multimer of the 72 kDa one. Deglycosylation of the 205 kDa and 72 kDa glycoproteins with trifluoromethanesulfonic acid yielded a 54 kDa polypeptide in both cases. At least three species of oligosaccharides were O-glycosidically linked to serines of the 54 kDa polypeptide chain. One of the oligosaccharides had N-acetylgalactosamine at the reducing end, rhamnose and a component not yet identified.

Gerwig, G. J., P. de Waard, et al. (1989). "Novel O-linked carbohydrate chains in the cellulase complex (cellulosome) of Clostridium thermocellum. 3-O-Methyl-N-acetylglucosamine as a constituent of a glycoprotein." J Biol Chem **264**(2): 1027-35.

Alkaline borohydride treatment of the cellulosome of Clostridium thermocellum yielded two major oligosaccharide-alditols, namely D-Galp-beta(1----4)-D-GalOH and (formula; see text) The compounds, isolated via gel permeation chromatography and high performance liquid chromatography, were analyzed by monosaccharide analysis, methylation analysis, gas-liquid chromatography/mass spectrometry, fast atom bombardment/mass spectrometry, and one- and two-dimensional 500-MHz (COSY, HOHAHA, ROESY) 1H NMR spectroscopy. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis combined with blotting technology indicated that the tetrasaccharide is mainly associated with one of the cellulosome subunits.

Gerwig, G. J., J. P. Kamerling, et al. (1991). "Primary structure of O-linked carbohydrate chains in the cellulosome of different Clostridium thermocellum strains." Eur J Biochem **196**(1): 115-22.

The cell-free forms of the multiple cellulase-containing protein complex (cellulosome), isolated from the cellulolytic bacterium Clostridium thermocellum strains YS, ATCC 27405 and LQRI, have a total carbohydrate content of 5-7% (by mass), consisting of O-linked oligosaccharide chains. The carbohydrate chains were liberated by alkaline-borohydride treatment and fractionated as oligosaccharide alditols via gel-permeation chromatography and HPLC. The fractions were investigated by 500-MHz 1H-NMR spectroscopy in combination with monosaccharide and methylation analysis and with fast-atom-bombardment

mass spectrometry (FAB-MS). In addition to the previously described major oligosaccharide, (formula; see text) [Gerwig, G. J., de Waard, P., Kamerling, J. P., Vliegenthart, J. F. G., Morgenstern, E., Lamed, R. & Bayer, E. A. (1989) J. Biol. Chem. 264, 1027-1035], the following partial structures of this compound could be established: (formula; see text). Cell-free and cell-associated forms of the cellulosome of C. thermocellum, as determined for strain YS, have the same oligosaccharide pattern. Based on the oligosaccharide structures, a biosynthetic pathway is suggested.

Gerwig, G. J., J. P. Kamerling, et al. (1992). "Novel oligosaccharide constituents of the cellulase complex of Bacteroides cellulosolvens." Eur J Biochem **205**(2): 799-808.

The multiple cellulase-containing protein complex, isolated from the cellulolytic bacterium Bacteroides cellulosolvens, contains oligosaccharides which are O-linked mainly to a 230-kDa subunit. The oligosaccharide chains were liberated by alkaline-borohydride treatment and fractionated as oligosaccharide alditols via gel-permeation chromatography and HPLC. The fractions were investigated by one- and two-dimensional (correlation, homonuclear Hartmann-Hahn, rotating-frame nuclear Overhauser enhancement) 500-MHz 1H-NMR spectroscopy in combination with monosaccharide and methylation analyses and with fast-atom-bombardment mass spectrometry. The following carbohydrate structures could be established: [formula: see text] The results indicate an interesting similarity between the oligosaccharide moieties of the cellulase complex of B. cellulosolvens and of Clostridium thermocellum [Gerwig, G. J., Kamerling, J. P., Vliegenthart, J. F. G., Morag (Morgenstern), E., Lamed, R. & Bayer, E. A. (1991) Eur. J. Biochem. 196, 115-122], having 3, 5 and 6 as common elements. The furanose form of a terminal alpha-D-galactose residue demonstrated an inhibitory effect on the interaction of Griffonia simplicifolia I isolectin B4 with the cellulosome-like entity of B. cellulosolvens.

Gerwig, G. J., J. P. Kamerling, et al. (1993). "The nature of the carbohydrate-peptide linkage region in glycoproteins from the cellulosomes of Clostridium thermocellum and Bacteroides cellulosolvens." J Biol Chem **268**(36): 26956-60.

The cellulase complexes of two cellulolytic bacteria, Clostridium thermocellum and Bacteroides cellulosolvens, were subjected to extensive Pronase digestion. Glycopeptide fractions were isolated by gel permeation and fast protein liquid chromatography and analyzed by monosaccharide analysis, amino acid analysis, methylation analysis, and 1H NMR spectroscopy. Alkaline borohydride-induced deglycosylation/amino acid conversion and periodate oxidation studies on the glycopeptide fraction of the C. thermocellum cellulosome demonstrated that the earlier established collection of carbohydrate moieties with 3-O-Me-D-GlcpNAc-alpha (1-->2)-[D-Galp-alpha (1-->3)]-D-Galf-alpha (1-->2)-D-Gal (where 3-O-Me-D-GlcpNAc is 3-O-methyl-N-acetylglucopyranosamine, Galp is galactopyranose, and Galf is galactofuranose) as the major component, is O-linked to threonine via galactopyranose. Using the same approach for the glycopeptide fraction of the cellulase complex of B. cellulosolvens, it was found that the reported collection of carbohydrate moieties with D-Galf-alpha (1-->3)-D-GlcpNAc-alpha (1-->2)-D-

Galf-alpha (1-->2)-[D-Galf-beta (1-->3)]-D-Gal as the major component, is O-linked mainly to threonine and partly to serine via galactopyranose. In both species, the hydroxyamino-acid-bound galactopyranose residue has probably an alpha-configuration. The carbohydrate chains appear as clusters located in highly Thr/Pro-rich peptide regions of the glycoproteins. The results are consistent with the notion that the glycosylation sites are localized in linker sequences which connect the various binding domains of the noncatalytic S1 subunit of the cellulosome.

Gilkes, N. R., M. L. Langsford, et al. (1984). "Mode of action and substrate specificities of cellulases from cloned bacterial genes." J Biol Chem **259**(16): 10455-9.

Three recombinant plasmids, pEC1, pEC2, and pEC3, each containing a unique Cellulomonas fimi chromosomal DNA insert, expressed Cm-cellulase activities in Escherichia coli C600 (Whittle, D. J., Kilburn, D. H., Warren, R. A. J., and Miller, R. C., Jr. (1982) Gene (Amst.) 17, 139-145; Gilkes, N. R., Kilburn, D. G., Langsford, M. L., Miller, R. C., Jr., Wakarchuk, W. W., Warren, R. A. J., Whittle, D. J., and Wong, W. K. R. (1984) J. Gen. Microbiol. 130, 1377-1384). Viscometric and chemical analyses showed that the enzymes encoded by pEC2 and pEC3 behaved as endoglucanases, whereas that encoded by pEC1 behaved as an exoglucanase. The activities of the exoglucanase and the pEC2-encoded endodglucanase were additive on Cm-cellulose as substrate. The pEC1-encoded enzyme also hydrolyzed xylan and p-nitrophenyl cellobioside. Two substrate-bound Cm-cellulases were isolated from the residual cellulose in a C. fimi culture by guanidine hydrochloride elution, affinity chromatography, and polyacrylamide gel electrophoresis. Both were glycoproteins of apparent Mr = 58,000 and 56,000, respectively. The 56-kDa enzyme appeared to be identical with the pEC1-encoded product, suggesting that they arise from the same gene.

Goldman, S., K. Hecht, et al. (1990). "Extracellular Ca2(+)-dependent inducible alkaline phosphatase from extremely halophilic archaebacterium Haloarcula marismortui." J Bacteriol **172**(12): 7065-70.

When starved of inorganic phosphate, the extremely halophilic archaebacterium Haloarcula marismortui produces the enzyme alkaline phosphatase and secretes it to the medium. This inducible extracellular enzyme is a glycoprotein whose subunit molecular mass is 160 kDa, as estimated by sodium dodecyl sulfate-gel electrophoresis. The native form of the enzyme is heterogeneous and composed of multiple oligomeric forms. The enzymatic activity of the halophilic alkaline phosphatase is maximal at pH 8.5, and the enzyme is inhibited by phosphate. Unlike most alkaline phosphatases, the halobacterial enzyme requires Ca2+ and not Zn2+ ions for its activity. Both calcium ions (in the millimolar range) and NaCl (in the molar range) are required for the stability of the enzyme.

Gonzalez-Zamorano, M., G. Mendoza-Hernandez, et al. (2009). "Mycobacterium tuberculosis glycoproteomics based on ConA-lectin affinity capture of mannosylated proteins." J Proteome Res **8**(2): 721-33.

A Mycobacterium tuberculosis culture filtrate enriched with mannose-containing

proteins was resolved by 2-DE gel. After ConA ligand blotting, 41 proteins were identified by mass spectrometry as putative glycoproteins with 34 of them new probably mannosylated proteins. These results contribute to the construction of the ConA affinity glycoprotein database of M. tuberculosis, and provide useful information for understanding the biological role of glycoproteins in mycobacteria.

Grass, S., A. Z. Buscher, et al. (2003). "The Haemophilus influenzae HMW1 adhesin is glycosylated in a process that requires HMW1C and phosphoglucomutase, an enzyme involved in lipooligosaccharide biosynthesis." Mol Microbiol **48**(3): 737-51.

Non-typeable Haemophilus influenzae is a common respiratory pathogen and an important cause of morbidity in humans. The non-typeable H. influenzae HMW1 and HMW2 adhesins are related proteins that mediate attachment to human epithelial cells, an essential step in the pathogenesis of disease. Secretion of these adhesins requires accessory proteins called HMW1B/HMW2B and HMW1C/HMW2C. In the present study, we investigated the specific function of HMW1C. Examination of mutant constructs demonstrated that HMW1C influences both the size and the secretion of HMW1. Co-immunoprecipitation and yeast two-hybrid assays revealed that HMW1C interacts with HMW1 and forms a complex in the cytoplasm. Additional experiments and homology analysis established that HMW1C is required for glycosylation of HMW1 and may have glycotransferase activity. The glycan structure contains galactose, glucose and mannose and appears to be generated in part by phosphoglucomutase, an enzyme important for lipooligosaccharide biosynthesis. In the absence of glycosylation, HMW1 is partially degraded and is efficiently released from the surface of the organism, resulting in reduced adherence. Based on these results, we conclude that glycosylation is a prerequisite for HMW1 stability. In addition, glycosylation appears to be essential for optimal HMW1 tethering to the bacterial surface, which in turn is required for HMW1-mediated adherence, thus revealing a novel mechanism by which glycosylation influences cell-cell interactions.

Greller, G., R. Riek, et al. (2001). "Purification and characterization of the heterologously expressed trehalose/maltose ABC transporter complex of the hyperthermophilic archaeon Thermococcus litoralis." Eur J Biochem **268**(14): 4011-8.

We report the purification of the maltose/trehalose transporter complex MalFGK of the hyperthermophilic archaeon Thermococcus litoralis. The complex was expressed in Escherichia coli, solubilized in dodecyl maltoside and purified with the aid of a histidine tag on one of the membrane proteins. One hundred grams of cells yielded 3 mg of pure complex. The final product showed ATPase activity at 70 degrees C and was soluble at low detergent concentration. ATPase activity was not due to dissociation of the MalK subunit from the integral membrane proteins MalF and MalG but could not be further stimulated by trehalose/maltose binding protein (TMBP), be it the native protein as isolated from T. litoralis or the soluble engineered protein. The purified native TMBP was identified as a glycoprotein.

Grogan, D. W. (1989). "Phenotypic characterization of the archaebacterial genus

Sulfolobus: comparison of five wild-type strains." J Bacteriol **171**(12): 6710-9.

Though amenable to routine manipulation and a popular subject of molecular genetic and biochemical studies on archaebacteria, the genus Sulfolobus has remained poorly described in phenotypic terms. To delineate their physiological capabilities and diversity, five laboratory strains, including type strains of the described species Sulfolobus acidocaldarius and S. solfataricus, were compared with respect to a variety of growth and biochemical parameters, including component profile of the surface-layer cell wall, inhibitors of growth, growth rate as a function of temperature and pH, and compounds used as sole sources of carbon or nitrogen. Motility and photoregulated production of an orange pigment were detected in all five strains tested. The results provide new criteria for distinguishing Sulfolobus strains as well as potential tools for the physiological and genetic manipulation of these extreme thermophiles.

Guerry, P., C. P. Ewing, et al. (2007). "Protein glycosylation in Campylobacter jejuni: partial suppression of pglF by mutation of pseC." J Bacteriol **189**(18): 6731-3.

Campylobacter jejuni has systems for N- and O-linked protein glycosylation. Although biochemical evidence demonstrated that a pseC mutant in the O-linked pathway accumulated the product of pglF in the N-linked pathway, analyses of transformation frequencies and glycosylation statuses of N-glycosylated proteins indicated a partial suppression of pglF by pseC.

Hanna, E. S., M. C. Roque-Barreira, et al. (2007). "Evidence for glycosylation on a DNA-binding protein of Salmonella enterica." Microb Cell Fact **6**: 11.

BACKGROUND: All organisms living under aerobic atmosphere have powerful mechanisms that confer their macromolecules protection against oxygen reactive species. Microorganisms have developed biomolecule-protecting systems in response to starvation and/or oxidative stress, such as DNA biocrystallization with Dps (DNA-binding protein from starved cells). Dps is a protein that is produced in large amounts when the bacterial cell faces harm, which results in DNA protection. In this work, we evaluated the glycosylation in the Dps extracted from Salmonella enterica serovar Typhimurium. This Dps was purified from the crude extract as an 18-kDa protein, by means of affinity chromatography on an immobilized jacalin column. RESULTS: The N-terminal sequencing of the jacalin-bound protein revealed 100% identity with the Dps of S. enterica serovar Typhimurium. Methyl-alpha-galactopyranoside inhibited the binding of Dps to jacalin in an enzyme-linked lectin assay, suggesting that the carbohydrate recognition domain (CRD) of jacalin is involved in the interaction with Dps. Furthermore, monosaccharide compositional analysis showed that Dps contained mannose, glucose, and an unknown sugar residue. Finally, jacalin-binding Dps was detected in larger amounts during the bacterial earlier growth periods, whereas high detection of total Dps was verified throughout the bacterial growth period. CONCLUSION: Taken together, these results indicate that Dps undergoes post-translational modifications in the pre- and early stationary phases of bacterial growth. There is also evidence that a small mannose-containing oligosaccharide is linked to this bacterial protein.

Hartmann, M., A. Barsch, et al. (2004). "The glycosylated cell surface protein Rpf2, containing a resuscitation-promoting factor motif, is involved in intercellular communication of Corynebacterium glutamicum." Arch Microbiol **182**(4): 299-312.

The genome of Corynebacterium glutamicum ATCC 13032 contains two genes, rpf1 and rpf2, encoding proteins with similarities to the essential resuscitation-promoting factor (Rpf) of Micrococcus luteus. Both the Rpf1 (20.4 kDa) and Rpf2 (40.3 kDa) proteins share the so-called Rpf motif, a highly conserved protein domain of approximately 70 amino acids, which is also present in Rpf-like proteins of other gram-positive bacteria with a high G+C content of the chromosomal DNA. Purification of the C. glutamicum Rpf2 protein from concentrated supernatants, SDS-PAGE and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identified modified Rpf2 variants with increased or reduced mobility when compared with the calculated size of Rpf2. A Western blot-based enzyme immunoassay demonstrated glycosylation of the Rpf2 variants with higher molecular masses. Galactose and mannose were identified as two components of the oligosaccharide portion of the Rpf2 glycoprotein by capillary gas chromatography coupled to mass spectrometry. The Rpf2 protein was localized on the surface of C. glutamicum with the use of immuno-fluorescence microscopy. C. glutamicum strains with defined deletions in the rpf1 or rpf2 gene or simultaneous deletions in both rpf genes were constructed, indicating that the rpf genes are neither individually nor collectively essential for C. glutamicum. The C. glutamicum rpf double mutant displayed slower growth and a prolonged lag phase after transfer of long-stored cells into fresh medium. The addition of supernatant from exponentially growing cultures of the rpf double mutant, the wild type or C. glutamicum strains with increased expression of the rpf1 or rpf2 gene significantly reduced the lag phase of long-stored wild-type and rpf single mutant strains, but addition of purified His-tagged Rpf1 or Rpf2 did not. In contrast, the lag phase of the C. glutamicum rpf double mutant was not affected upon addition of these culture supernatants.

Herrmann, J. L., R. Delahay, et al. (2000). "Analysis of post-translational modification of mycobacterial proteins using a cassette expression system." FEBS Lett **473**(3): 358-62.

A recombinant expression system was developed to analyse sequence determinants involved in O-glycosylation of proteins in mycobacteria. By expressing peptide sequences corresponding to known glycosylation sites within a chimeric lipoprotein construct, amino acids flanking modified threonine residues were found to have an important influence on glycosylation. The expression system was used to screen mycobacterial sequences selected using a neural network (NetOglyc) trained on eukaryotic O-glycoproteins. Evidence of glycosylation was obtained for eight of 11 proteins tested. The results suggest that sites involved in O-glycosylation of mycobacterial and eukaryotic proteins share similar structural features.

Herzberg, M. C., P. R. Erickson, et al. (1990). "Platelet-interactive products of Streptococcus sanguis protoplasts." Infect Immun **58**(12): 4117-25.

To isolate a more native, platelet-interactive macromolecule (class II antigen) of Streptococcus sanguis, cultured protoplasts were used as a source. Protoplasts were optimally prepared from fresh washed cells by digestion with 80 U of mutanolysin per ml for 75 min at 37 degrees C while osmotically stabilized in 26% (wt/vol) raffinose. Osmotically stabilized forms were surrounded by a 9-nm bilaminar membrane, as shown by transmission electron microscopy. Protoplasts were cultured in chemically defined synthetic medium and osmotically stabilized by ammonium chloride. Spent culture media were harvested daily for 7 days. Each day, soluble proteins were isolated from media, preincubated with platelet-rich plasma, and tested for inhibition of platelet aggregation induced by S. sanguis cells. Products released from S. sanguis protoplasts and reactive with an anti-class II antigen immunoaffinity matrix were able to inhibit S. sanguis-induced platelet aggregation. As resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, anti-class II-reactive protoplast products included silver-stained bands of 67, 79, 115, 216, and 248 kDa. The 115-kDa protein fraction was isolated by gel filtration and ion-exchange chromatography. This form of the class II antigen contained N-formylmethionine at its amino terminus. Rhamnose constituted 18.2% of the total residual dry weight and nearly half of its carbohydrate content. Diester phosphorus constituted 1% of this fraction. After trypsinization of the protoplast products from either preparation, a 65-kDa protein fragment was recovered. This protoplast protein fragment and the S. sanguis cell-derived 65-kDa class II antigen, previously implicated in the induction of platelet aggregation, were shown to be functionally and immunologically identical.

Hirai, H., R. Takai, et al. "Glycosylation regulates the specific induction of rice immune responses by Acidovorax avenae flagellin." J Biol Chem.

Plants have a sensitive system that detects various pathogen-derived molecules to protect against infection. Flagellin, a main component of the bacterial flagellum, from the rice avirulent N1141 strain of gram-negative phytopathogenic bacterium, Acidovorax avenae, induces plant immune responses including H(2)O(2) generation, while flagellin from the rice virulent K1 strain of A. avenae does not induce these immune responses. To clarify the molecular mechanism that leads to these differing responses between the K1 and N1141 flagellins, recombinant K1 and N1141 flagellins were generated using an Escherichia coli expression system. When cultured rice cells were treated with recombinant K1 or N1141 flagellin, both flagellins equally induced H(2)O(2) generation, suggesting that post-translational modifications of the flagellins are involved in the specific induction of immune responses. Mass spectrometry analyses using glycosyltransferase-deficient mutants showed that 1,600 Da and 2,150 Da glycans were present on the flagellins from N1141 and K1, respectively. A deglycosylated K1 flagellin induced immune responses in the same manner as N1141 flagellin. Site-directed mutagenesis revealed that glycans were attached to four amino acid residues ((178)Ser, (183)Ser, (212)Ser and (351)Thr) in K1 flagellin. Among mutant K1 flagellins in which each glycan-attached amino acid residue was changed to alanine, (178)Ser/Ala and (183)Ser/Ala K1 flagellin

induced a strong immune response in cultured rice cells, indicating that the glycans at (178)Ser and (183)Ser in K1 flagellin prevent epitope recognition in rice.

Hoiczyk, E. and W. Baumeister (1997). "Oscillin, an extracellular, Ca2+-binding glycoprotein essential for the gliding motility of cyanobacteria." Mol Microbiol **26**(4): 699-708.

Electron microscopic studies have demonstrated that various gliding filamentous cyanobacteria have trichome surfaces with a common structural organization. They contain an S-layer attached to the outer membrane and an array of parallel fibrils on top of the S-layer. In all species studied, the helical arrangement of these fibrils corresponds to the sense of rotation of the organism during the gliding movement. We have investigated the surface fibrils of Phormidium uncinatum using electron microscopic, spectroscopic and biochemical techniques. The fibrils consist of a single rod-shaped protein, which we refer to as oscillin. Oscillin is a 646 amino acid residue protein (Mr 65807; pI 3.63) and appears to be glycosylated. Sequence analysis reveals a two-domain structure: a 554 residue domain contains 46 repeats of a Ca2+-binding motif; it is followed by a 92 residue C-terminal domain, which might mediate its export. Filaments that do not express oscillin lose their ability to move. Homology studies suggest that similar proteins play comparable roles in other motile cyanobacteria. The structure of oscillin appears to favour a passive role in gliding.

Huang, L., C. W. Forsberg, et al. (1988). "Purification and characterization of a chloride-stimulated cellobiosidase from Bacteroides succinogenes S85." J Bacteriol **170**(7): 2923-32.

A cellobiosidase with unique characteristics from the extracellular culture fluid of the anaerobic gram-negative cellulolytic rumen bacterium Bacteroides succinogenes grown on microcrystalline cellulose (Avicel) in a continuous culture system was purified to homogeneity by column chromatography. The enzyme was a glycoprotein with a molecular weight of approximately 75,000 and an isoelectric point of 6.7. When assayed at 39 degrees C and pH 6.5, the activity of the enzyme with p-nitrophenyl-beta-D-cellobioside as the substrate was stimulated by chloride, bromide, fluoride, iodide, nitrate, and nitrite, with maximum activation (approximately sevenfold) occurring at concentrations ranging from 1.0 mM (Cl-) to greater than 0.75 M (F-). The presence of chloride (0.2 M) did not affect the Km but doubled the Vmax. In the presence of chloride (0.2 M), the pH optimum of the enzyme was broadened, and the temperature optimum was increased from 39 to 45 degrees C. The enzyme released terminal cellobiose from cellotriose and cellobiose and cellotriose from longer-chain-length cellooligosaccharrides and acid-swollen cellulose, but it had no activity on cellobiose. The enzyme showed affinity for cellulose (Avicel) but did not hydrolyze it. It also had a low activity on carboxymethyl cellulose.

Iki, K., K. Kawahara, et al. (1997). "A novel component different from endotoxin extracted from Prevotella intermedia ATCC 25611 activates lymphoid cells from

C3H/HeJ mice and gingival fibroblasts from humans." Infect Immun **65**(11): 4531-8.
A novel immunobiologically active fraction was prepared from a phenol-water extract of Prevotella intermedia ATCC 25611 by Sephadex G-100 column chromatography. The fraction consisted mainly of carbohydrate and protein and was devoid of fatty acid. The fraction showed high-molecular-weight bands (10,000 to 12,000) on deoxycholate polyacrylamide gel electrophoresis (DOC-PAGE) and was scarcely active in a Limulus test. We designated the fraction Prevotella glycoprotein (PGP). The PGP fraction showed strong mitogenicity on splenocytes and cytokine-inducing activities on peritoneal macrophages from both C3H/HeJ and C3H/HeN mice, and it stimulated human gingival fibroblasts to produce cytokines. The activities of the PGP fraction were resistant to heat inactivation (100 degrees C for 1 h) and protease treatments and were scarcely inhibited by polymyxin B. In contrast, the purified lipopolysaccharide fraction (LPS-PCP) extracted from the same bacterium with a phenol-chloroform-petroleum ether mixture, which showed strong Limulus activity and a single low-molecular-weight band (approximately 3,000) on DOC-PAGE, lacked the activities on splenocytes and macrophages from C3H/HeJ mice and human gingival fibroblasts. The activities of the LPS-PCP fraction on cells from C3H/HeN mice were completely inhibited by polymyxin B. The LPS extracted from the same bacterium with hot phenol-water (LPS-PW) exhibited the properties of both the PGP fraction and the LPS-PCP fraction. These findings suggest that the unique bioactivities of the LPS-PW fraction of oral black-pigmented bacteria reported to date, which differed from those of the classical endotoxin, were derived from the PGP fraction and not from the LPS itself.

Jarrell, K. F., D. P. Bayley, et al. (1996). "The archaeal flagellum: a unique motility structure." J Bacteriol **178**(17): 5057-64.

Josenhans, C., R. L. Ferrero, et al. (1999). "Cloning and allelic exchange mutagenesis of two flagellin genes of Helicobacter felis." Mol Microbiol **33**(2): 350-62.
Helicobacter felis has been used extensively in animal model studies of gastric Helicobacter infections. Attempts to manipulate H. felis genetically have, however, been unsuccessful and, consequently, little is known about the pathogenic mechanisms of this bacterium. In common with other Helicobacter spp., H. felis is a highly motile organism. To characterize the flagellar structures responsible for this motility, we cloned and sequenced the two flagellin-encoding genes, flaA and flaB, from H. felis. These genes encode two flagellin proteins that are expressed simultaneously under the control of putative sigma28 and sigma54 promoters respectively. Isogenic mutants of H. felis in flaA and flaB were generated by electroporation-mediated allelic disruption and replacement, showing for the first time that H. felis could be manipulated genetically. Both types of H. felis flagellin mutants exhibited truncated flagella and were poorly motile. H. felis flaA mutants were unable to colonize the gastric mucosa in a mouse infection model.

Josenhans, C., L. Vossebein, et al. (2002). "The neuA/flmD gene cluster of Helicobacter

pylori is involved in flagellar biosynthesis and flagellin glycosylation." FEMS Microbiol Lett **210**(2): 165-72.

Helicobacter pylori possesses a gene (HP0326/JHP309) homologous to neuA of other bacteria, encoding a cytidyl monophosphate-N-acetylneuraminic acid synthetase-homologous enzyme in its N-terminal portion. We analysed the function of this gene, which is controlled by a flagellar class 2 sigma(54) promoter, in flagellar biosynthesis. HP0326/JHP309 actually represents a bicistronic operon consisting of a neuA and a flmD-like putative glycosyl transferase gene. An isogenic flmD mutant synthesized basal bodies but no filaments, was non-motile, and expressed severely reduced amounts of a FlaA flagellin of reduced molecular mass. FlaA flagellin was found to be glycosylated in its exported form within the flagellar filament, but not inside the cytoplasm. Glycosylated FlaA was not detectable in the flmD mutant. Together with other genes in the H. pylori genome, a proposed function of the neuA/flmD gene products could be to provide a pathway for glycosylation of flagellin and other extracytoplasmic molecules during type III secretion.

Kanaoka, M., Y. Fukita, et al. (1987). "Antitumor activity of streptococcal acid glycoprotein produced by Streptococcus pyogenes Su." Jpn J Cancer Res **78**(12): 1409-14.

Streptococcal acid glycoprotein (SAGP) was purified from the cultured cells of Streptococcus pyogenes Su, and its in vitro and in vivo antitumor activities were investigated in comparison with those of OK-432, a cell preparation of S. pyogenes Su which is used clinically as a potent antitumor agent. SAGP inhibited the growth of several tumor cell lines in vitro at less than 0.1 microgram/ml, while it did not affect the growth of the other tumor and normal cell lines even at 10 micrograms/ml. This selective cytotoxicity is a unique characteristic of SAGP. OK-432 did not show cytotoxicity in vitro. SAGP also showed a considerable life-span-prolonging effect on mice bearing Meth A tumor and inhibited the growth of sarcoma 180 tumor implanted im. The comparison of antitumor activities between SAGP and OK-432 definitely suggested a difference in the mechanisms of their actions, even though they were derived from the same bacterial strain.

Kanaoka, M., T. Negoro, et al. (1991). "Streptococcal antitumor protein: expression in Escherichia coli cells and properties of the recombinant protein." Agric Biol Chem **55**(3): 743-50.

Streptococcal antitumor protein (SAGP) was produced by transformed E. coli JM103 carrying the SAGP gene downstream from the tac promoter. The purified recombinant SAGP had the same N-terminal amino acid sequence as that of the native SAGP isolated from Streptococcus pyogenes Su cells. Gel filtration analysis showed that the recombinant SAGP was a dimer, while the native SAGP was a tetramer. When the antitumor activity was tested against sarcoma 180 cells, the IC50 of the recombinant SAGP was 0.3 microgram/ml, about a quarter as active as the native SAGP. These results suggest that the quaternary structure of SAGP is important for the antitumor activity.

Karjalainen, T., A. J. Waligora-Dupriet, et al. (2001). "Molecular and genomic analysis of genes encoding surface-anchored proteins from Clostridium difficile." Infect Immun **69**(5): 3442-6.

The gene slpA, encoding the S-layer precursor protein in the virulent Clostridium difficile strains C253 and 79--685, was identified. The precursor protein carries a C-terminal highly conserved anchoring domain, similar to the one found in the Cwp66 adhesin (previously characterized in strain 79--685), an SLH domain, and a variable N-terminal domain mediating cell adherence. The genes encoding the S-layer precursor proteins and the Cwp66 adhesin are present in a genetic locus carrying 17 open reading frames, 11 of which encode a similar two-domain architecture, likely to include surface-anchored proteins.

Kawamura, T. and G. D. Shockman (1983). "Purification and some properties of the endogenous, autolytic N-acetylmuramoylhydrolase of Streptococcus faecium, a bacterial glycoenzyme." J Biol Chem **258**(15): 9514-21.

The latent form of the endogenous, autolytic N-acetylmuramoylhydrolase of Streptococcus faecium ATCC 9790 was purified to near homogeneity by affinity chromatography on concanavalin A-Sepharose 4B. The latent enzyme had Mr approximately 130,000 on sodium dodecyl sulfate-gel electrophoresis. Upon proteinase treatment (trypsin or endogenous proteinase), the latent form is converted to an active form Mr approximately 87,000. The enzyme was shown to be glycoprotein, containing monomeric and oligomeric glucose substituents. Some of the substrate specificity requirements of this enzyme are described.

Khmelenina, V. N., M. G. Kalyuzhnaya, et al. (1999). "Osmoadaptation in halophilic and alkaliphilic methanotrophs." Arch Microbiol **172**(5): 321-9.

By using (1)H- and (13)C-NMR spectroscopy, an accumulation of sucrose and two cyclic amino acids [ectoine (1,4,5, 6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) and 5-oxoproline (pyrrolidone carboxylic acid)] was detected in the halotolerant methanotrophs Methylobacter alcaliphilus 20Z and Methylobacter modestohalophilus 10S. The organic solute pool was found to increase upon raising the NaCl concentration. In M. alcaliphilus 20Z, the intracellular level of the total solutes was shown to be sufficient to balance the osmotic pressure of the medium, whereas in M. modestohalophilus 10S their content was several times lower. Additionally, phosphatidylglycerol and phosphatidylcholine were predominant cell phospholipids in salt-adapted M. alcaliphilus 20Z. However, no phosphatidylcholine was found in M. modestohalophilus 10S, and the portion of phosphatidylglycerol increased while phosphatidylethanolamine decreased upon elevated external NaCl concentrations. Regularly arranged glycoprotein surface layers (S-layers) of hexagonal and linear (p2) symmetry were observed on the outer cell walls of M. alcaliphilus 20Z and M. modestohalophilus 10S. The S-layer in M alcaliphilus 20Z consisting of tightly packed, cup-shaped subunits was lost during growth at pH 7.2 (the lowest possible pH) in the absence of NaCl. Hence, osmoadaptation in the methanotrophs studied involves structure/function alterations of cell envelopes and changes in the chemical composition of membranes as well as de

novo synthesis of compatible solutes.

Kim, B. K., T. D. Pihl, et al. (1995). "Purification of the copper response extracellular proteins secreted by the copper-resistant methanogen Methanobacterium bryantii BKYH and cloning, sequencing, and transcription of the gene encoding these proteins." J Bacteriol **177**(24): 7178-85.

When the copper-resistant methanogen Methanobacterium bryantii BKYH was exposed to 1 mM Cu(II), it secreted approximately fourfold increased levels of three proteins, copper response extracellular (CRX) proteins. The members of the CRX protein trio had apparent molecular masses of 40.8, 42.3, and 42.9 kDa and were purified together from the culture supernatant and separated from each other by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The N-terminal amino acid sequences of the three proteins were essentially identical, and antibodies raised against one of the trio reacted with all three proteins and with three other intracellular proteins with slightly higher molecular weights. The N-terminal amino acid sequence of one of these larger proteins was different from that of the secreted CRX proteins. The gene crx, which encodes the CRX proteins, was cloned and sequenced, and crx transcription was characterized. The crx sequence predicts that the encoded polypeptide is synthesized as a precursor with an N-terminal leader peptide, containing 28 amino acid residues, that is removed during the extracellular secretion of the CRX proteins. Transcription was initiated 274 bp upstream from the crx gene, producing an approximately 1.4-kb monocistronic transcript that was present in M. bryantii BKYH cells under all growth conditions but that increased approximately fourfold in vivo in response to Cu addition. The CRX proteins appear to be glycosylated, since they react with concanavalin A and neuraminidase, and to be the products of one gene that have different levels of posttranslational glycosylation. This is supported by very similar chromatographic and electrophoretic properties, identical N-terminal amino acid sequences, immunological cross-reactivities, and the detection of only one crx-related sequence by Southern blotting. Western blots (immunoblots) showed no evidence for CRX proteins in cell lysates of several other Methanobacterium strains.

Kluepfel, D., S. Vats-Mehta, et al. (1990). "Purification and characterization of a new xylanase (xylanase B) produced by Streptomyces lividans 66." Biochem J **267**(1): 45-50.

A new extracellular xylanase produced by Streptomyces lividans 66 was isolated from a genetically engineered clone of that strain. This enzyme, named xylanase B, has an Mr of 31,000 and acts specifically on xylan as an endo-type xylanase producing short-chain xylo-oligosaccharides. The activity is optimal at pH 6.5 and at a temperature of 55 degrees C, which is similar to that of the previously characterized xylanase A. Xylanase B is glycosylated and has a pI of 8.4; its Km and Vmax. values are 3.71 mg/ml and 1.96 mmol/mg of enzyme respectively. Specific antibodies raised against xylanase A show no cross-reaction with xylanase B; however, the anti-(xylanase B) antibodies react slightly with xylanase A. A comparison of the hydrolysis products obtained from oat-spelts xylan with

both enzymes show that xylanase A preferentially degrades short-chain oligo-xylosides, whereas xylanase B acts on the longer, water-insoluble, molecules.

Kondo, E., T. Kurata, et al. (1996). "Evolution of cell-surface acid phosphatase of Burkholderia pseudomallei." Southeast Asian J Trop Med Public Health **27**(3): 592-9.
Acid phosphatase active fractions were obtained from cell-free extract, outermembrane fraction and culture filtrate of Burkholderia pseudomallei by column chromatography with sepharose 6B and DEAE cellulose. The comparison of the elution patterns of protein, sugar and enzymatic activity among these three components suggested that the enzyme is a glycoprotein evolving from premature proteins through glycosylation and that the enzyme is translocated during glycosylation from the cytoplasm to the outer membrane and finally excreted into the environment. When tunicamycin, a glycosylation inhibitor, was added to the culture, the peaks of sugar and enzymatic activity were lowered concomitantly leaving the protein peak unchanged in the elution pattern of the culture filtrate. The affinity of the bacterial surface to antienzyme sera was demonstrated by immuno-fluorescence microscopy.

Koning, S. M., M. G. Elferink, et al. (2001). "Cellobiose uptake in the hyperthermophilic archaeon Pyrococcus furiosus is mediated by an inducible, high-affinity ABC transporter." J Bacteriol **183**(17): 4979-84.
The hyperthermophilic archaeon Pyrococcus furiosus can utilize different beta-glucosides, like cellobiose and laminarin. Cellobiose uptake occurs with high affinity (K(m) = 175 nM) and involves an inducible binding protein-dependent transport system. The cellobiose binding protein (CbtA) was purified from P. furiosus membranes to homogeneity as a 70-kDa glycoprotein. CbtA not only binds cellobiose but also cellotriose, cellotetraose, cellopentaose, laminaribiose, laminaritriose, and sophorose. The cbtA gene was cloned and functionally expressed in Escherichia coli. cbtA belongs to a gene cluster that encodes a transporter that belongs to the Opp family of ABC transporters.

Koning, S. M., W. N. Konings, et al. (2002). "Biochemical evidence for the presence of two alpha-glucoside ABC-transport systems in the hyperthermophilic archaeon Pyrococcus furiosus." Archaea **1**(1): 19-25.
The hyperthermophilic archaeon Pyrococcus furiosus can utilize different carbohydrates, such as starch, maltose and trehalose. Uptake of alpha-glucosides is mediated by two different, binding protein-dependent, ATP-binding cassette (ABC)-type transport systems. The maltose transporter also transports trehalose, whereas the maltodextrin transport system mediates the uptake of maltotriose and higher malto-oligosaccharides, but not maltose. Both transport systems are induced during growth on their respective substrates.

Konrad, Z. and J. Eichler (2002). "Protein glycosylation in Haloferax volcanii: partial characterization of a 98-kDa glycoprotein." FEMS Microbiol Lett **209**(2): 197-202.
The plasma membrane of Haloferax volcanii contains several glycoproteins, including a 98-kDa species. Using lectin-based chromatography, the glycoprotein

was isolated and partially characterized. Sequence comparison, based on antibody binding as well as one-dimensional peptide maps show that the 98-kDa glycoprotein is distinct from the S-layer glycoprotein, the major glycoprotein in H. volcanii. The 98-kDa glycoprotein can be further distinguished from the S-layer glycoprotein on the basis of membrane attachment. Unlike the S-layer glycoprotein, the 98-kDa glycoprotein is not associated with the membrane in a Mg2+-dependent manner. Both proteins, however, apparently rely on a similar mechanism of glycosylation, since neither was affected by treatment with bacitracin or tunicamycin, agents known to interfere with protein glycosylation in other species. Finally, the pattern of glycosylation of the 98-kDa glycoprotein is not shared by a 95-kDa glycoprotein of the related Haloferax mediterranei strain.

Kozloff, L. M., M. A. Turner, et al. (1991). "Formation of bacterial membrane ice-nucleating lipoglycoprotein complexes." J Bacteriol **173**(20): 6528-36.

The preliminary finding that nonprotein additions to the protein product of the ice-nucleating gene of Pseudomonas syringae or Erwinia herbicola are essential for ice nucleation at the warmest temperatures has led to experiments aimed at identifying possible linkages between the ice protein and the other components. It appears that the protein is coupled to various sugars through N- and O-glycan linkages. Mannose residues are apparently bound via an N-glycan bond to the amide nitrogen of one or more of the three essential asparagine residues in the unique amino-terminal portion of the protein. In turn, these mannose residues are involved in the subsequent attachment of phosphatidylinositol to the nucleation structure. This phosphatidylinositol-mannose-protein structure is the critical element in the class A nucleating structure. In addition to sugars attached to the asparagine residues, additional sugar residues appear to be attached by O-glycan linkages to serine and threonine residues in the primary repeating octapeptide, which makes up 70% of the total ice protein. These additional sugar residues include galactose and glucosamine and most likely additional mannose residues. These conclusions were based on (i) the changes in ice-nucleating activity due to the action of N- and O-glycanases, alpha- and beta-mannosidoses, and beta-galactosidase; (ii) immunoblot analyses of ice proteins in cell extracts after enzyme treatments; and (iii) the properties of transformed Ice+ Escherichia coli cells containing plasmids with defined amino-terminal and carboxyl-terminal deletions in the ice gene. Finally, evidence is presented that these sugar residues may play a role in aggregating the ice gene lipoglycoprotein compound into larger aggregates, which are the most effective ice nucleation structures.

Kuo, C., N. Takahashi, et al. (1996). "An N-linked high-mannose type oligosaccharide, expressed at the major outer membrane protein of Chlamydia trachomatis, mediates attachment and infectivity of the microorganism to HeLa cells." J Clin Invest **98**(12): 2813-8.

The structure of the carbohydrate of the 40-kD major outer membrane component of Chlamydia trachomatis and its role in defining infectivity of the organism were investigated. The oligosaccharides were released from the

glycoprotein by N-glycanase digestion, coupled to a 2-aminopyridyl residue, and subjected to two-dimensional sugar mapping technique. The major fractions consisted of "high-mannose type" oligosaccharides containing 8-9 mannose residues. Bi- and tri-antennary "complex type" oligosaccharides having terminal galactose were detected as minor components. These oligosaccharides were N-linked and contained no sialic acid. This structural profile is consistent with our previous characterization based on lectin-binding and glycosidase digestion. Functional specificity of identified chlamydial oligosaccharides was analyzed using glycopeptides fractionated from ovalbumin and structurally defined oligosaccharides from other sources. The glycopeptide fraction having high-mannose type oligosaccharide, as compared to those having complex or hybrid-type, showed a stronger inhibitory effect on attachment and infectivity of chlamydial organisms to HeLa cells. Among high-mannose type oligosaccharides, the strongest inhibition was observed with mannose 8 as compared with mannose 6, 7, or 9. These results indicate that a specific high-mannose type oligosaccharide linked to the major outer membrane protein of C. trachomatis mediates attachment and infectivity of the organism to HeLa cells.

Kus, J. V., J. Kelly, et al. (2008). "Modification of Pseudomonas aeruginosa Pa5196 type IV Pilins at multiple sites with D-Araf by a novel GT-C family Arabinosyltransferase, TfpW." J Bacteriol **190**(22): 7464-78.

Pseudomonas aeruginosa Pa5196 produces type IV pilins modified with unusual alpha1,5-linked d-arabinofuranose (alpha1,5-D-Araf) glycans, identical to those in the lipoarabinomannan and arabinogalactan cell wall polymers from Mycobacterium spp. In this work, we identify a second strain of P. aeruginosa, PA7, capable of expressing arabinosylated pilins and use a combination of site-directed mutagenesis, electrospray ionization mass spectrometry (MS), and electron transfer dissociation MS to identify the exact sites and extent of pilin modification in strain Pa5196. Unlike previously characterized type IV pilins that are glycosylated at a single position, those from strain Pa5196 were modified at multiple sites, with modifications of alphabeta-loop residues Thr64 and Thr66 being important for normal pilus assembly. Trisaccharides of alpha1,5-D-Araf were the principal modifications at Thr64 and Thr66, with additional mono- and disaccharides identified on Ser residues within the antiparallel beta sheet region of the pilin. TfpW was hypothesized to encode the pilin glycosyltransferase based on its genetic linkage to the pilin, weak similarity to membrane-bound GT-C family glycosyltransferases (which include the Mycobacterium arabinosyltransferases EmbA/B/C), and the presence of characteristic motifs. Loss of TfpW or mutation of key residues within the signature GT-C glycosyltransferase motif completely abrogated pilin glycosylation, confirming its involvement in this process. A Pa5196 pilA mutant complemented with other Pseudomonas pilins containing potential sites of modification expressed nonglycosylated pilins, showing that TfpW's pilin substrate specificity is restricted. TfpW is the prototype of a new type IV pilin posttranslational modification system and the first reported gram-negative member of the GT-C glycosyltransferase family.

Lewis, L. O., A. A. Yousten, et al. (1987). "Characterization of the surface protein layers of the mosquito-pathogenic strains of Bacillus sphaericus." J Bacteriol **169**(1): 72-9.

The protein surface layers on the cell walls of mosquito-pathogenic and nonpathogenic Bacillus sphaericus strains were studied by structural, biochemical, and serological methods. The surface structure of two representative insect-pathogenic strains had the form of a delicate linear array with a repeat interval of 5 nm. This was distinctly different from the tetragonal array of the P-1 strain in spacing and arrangement. The surface layers were composed of acidic glycoproteins with molecular weights ranging from approximately 133,000 to 155,000. Peptide mapping and serological analysis of the surface proteins revealed eight distinct groups among the pathogens. These groups were very similar to the groupings determined by flagellar-antigen serotyping and bacteriophage typing.

Li, Z., F. Dumas, et al. (1993). "A species-specific periplasmic flagellar protein of Serpulina (Treponema) hyodysenteriae." J Bacteriol **175**(24): 8000-7.

We have previously reported that a 46-kDa protein present in an outer membrane protein preparation seemed to be a species-specific antigen of Serpulina hyodysenteriae (Z. S. Li, N. S. Jensen, M. Belanger, M.-C. L'Esperance, and M. Jacques, J. Clin. Microbiol. 30:2941-2947, 1992). The objective of this study was to further characterize this antigen. A Western blot (immunoblot) analysis and immunogold labeling with a monospecific antiserum against this protein confirmed that the protein was present in all S. hyodysenteriae reference strains but not in the nonpathogenic organism Serpulina innocens. The immunogold labeling results also indicated that the protein was associated with the periplasmic flagella of S. hyodysenteriae. N-terminal amino acid sequencing confirmed that the protein was in fact a periplasmic flagellar sheath protein. The molecular mass of this protein, first estimated to be 46 kDa by Western blotting, was determined to be 44 kDa when the protein was evaluated more precisely by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the protein was glycosylated, as determined by glycoprotein staining and also by N-glycosidase F treatment. Five other periplasmic flagellar proteins of S. hyodysenteriae, which may have been the core proteins and had molecular masses of 39, 35, 32, 30, and 29 kDa, were antigenically related and cross-reacted with the periplasmic flagellar proteins of S. innocens. Finally, serum from a pig experimentally infected with S. hyodysenteriae recognized the 44-kDa periplasmic flagellar sheath protein. Our results suggest that the 44-kDa periplasmic flagellar sheath protein of S. hyodysenteriae is a species-specific glycoprotein antigen.

Lindenthal, C. and E. A. Elsinghorst (1999). "Identification of a glycoprotein produced by enterotoxigenic Escherichia coli." Infect Immun **67**(8): 4084-91.

Enterotoxigenic Escherichia coli (ETEC) strain H10407 is capable of invading epithelial cell lines derived from the human ileocecum and colon in vitro. Two separate chromosomally encoded invasion loci (tia and tib) have been cloned

from this strain. These loci direct nonadherent and noninvasive laboratory strains of E. coli to adhere to and invade cultured human intestinal epithelial cells. The tib locus directs the synthesis of TibA, a 104-kDa outer membrane protein that is directly correlated with the adherence and invasion phenotypes. TibA is synthesized as a 100-kDa precursor (preTibA) that must be modified for biological activity. Outer membranes of recombinant E. coli expressing TibA or preTibA were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted to nitrocellulose. The presence of glycoproteins was detected by oxidization of carbohydrates with periodate and labeling with hydrazide-conjugated digoxigenin. Only TibA could be detected as a glycoprotein. Complementation experiments with tib deletion mutants of ETEC strain H10407 demonstrate that the TibA glycoprotein is expressed in H10407, that the entire tib locus is required for TibA synthesis, and that TibA is the only glycoprotein produced by H10407. Protease treatment of intact H10407 cells removes the carbohydrates on TibA, suggesting that they are surface exposed. TibA shows homology with AIDA-I from diffuse-adhering E. coli and with pertactin precursor from Bordetella pertussis. Both pertactin and AIDA-I are members of the autotransporter family of outer membrane proteins and are afimbrial adhesins that play an important role in the virulence of these organisms. Analysis of the predicted TibA amino acid sequence indicates that TibA is also an autotransporter. Analysis of the tib locus DNA sequence revealed an open reading frame with similarity to RfaQ, a glycosyltransferase. The product of this tib locus open reading frame is proposed to be responsible for TibA modification. These results suggest that TibA glycoprotein acts as an adhesin that may participate in the disease process.

Logan, S. M. (2006). "Flagellar glycosylation - a new component of the motility repertoire?" Microbiology **152**(Pt 5): 1249-62.

The biosynthesis, assembly and regulation of the flagellar apparatus has been the subject of extensive studies over many decades, with considerable attention devoted to the peritrichous flagella of Escherichia coli and Salmonella enterica. The characterization of flagellar systems from many other bacterial species has revealed subtle yet distinct differences in composition, regulation and mode of assembly of this important subcellular structure. Glycosylation of the major structural protein, the flagellin, has been shown most recently to be an important component of numerous flagellar systems in both Archaea and Bacteria, playing either an integral role in assembly or for a number of bacterial pathogens a role in virulence. This review focuses on the structural diversity in flagellar glycosylation systems and demonstrates that as a consequence of the unique assembly processes, the type of glycosidic linkage found on archaeal and bacterial flagellins is distinctive.

Lower, B. H. and P. J. Kennelly (2002). "The membrane-associated protein-serine/threonine kinase from Sulfolobus solfataricus is a glycoprotein." J Bacteriol **184**(10): 2614-9.

Treatment of a sodium dodecyl sulfate-polyacrylamide gel with periodic acid-

Schiff (PAS) stain or blotting with Galanthus nivalis agglutinin revealed the presence of several glycosylated polypeptides in a partially purified detergent extract of the membrane fraction of Sulfolobus solfataricus. One of the glycoproteins comigrated with the membrane-associated protein-serine/threonine kinase from S. solfataricus, which had been radiolabeled by autophosphorylation with [(32)P]ATP in vitro. Treatment with a chemical deglycosylating agent, trifluoromethanesulfonic acid, abolished PAS staining and reduced the M(r) of the protein kinase from approximately 67,000 to approximately 62,000. Protein kinase activity also adhered to, and could be eluted from, agarose beads containing bound G. nivalis agglutinin. Glycosylation of the protein kinase implies that at least a portion of this integral membrane protein resides on the external surface of the cell membrane.

Ma, Y. and T. M. Daniel (1983). "Isolation, characterization, and specificity of a glycoprotein antigen from Mycobacterium kansasii." <u>Am Rev Respir Dis</u> **128**(6): 1059-64.

We have isolated in highly purified form and characterized a glycoprotein antigen from culture filtrates of Mycobacterium kansasii. Immunoelectrophoretic studies demonstrated that this antigen is present only in M. kansasii among 14 species of mycobacteria studied. It is a potent tuberculin skin test antigen, but skin test reactions to it in sensitized guinea pigs display only limited specificity. Enzyme-linked immunoabsorbent antibody assays of serum samples from patients suffering from diseases caused by M. kansasii, M. intracellulare, and M. tuberculosis display no specificity with this antigen. These findings are best explained by hypothesizing a single highly antigenic determinant that is present as a moiety on many mycobacterial antigens and is widely shared among mycobacteria.

Maeba, P. Y. (1986). "Isolation of a surface glycoprotein from Myxococcus xanthus." <u>J Bacteriol</u> **166**(2): 644-50.

The isolation of a glycoprotein from vegetative cells of Myxococcus xanthus is reported. The protein, abbreviated VGP, was first identified during a survey of surface proteins as a major protein that could be radioiodinated in vegetative, but not developing, cells (P.Y. Maeba, J. Bacteriol. 155:1033-1041, 1983). The protein was extracted from membranes with Triton X-100 and subsequently purified by DEAE-cellulose chromatography, chromatofocusing, and gel filtration. The protein has an Mr of approximately 74,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and an isoelectric point of 3.2 to 3.3. The carbohydrate moiety which made up approximately 13.5% of the weight of the VGP comprised primarily neutral sugars and smaller amounts of hexosamines and uronic acids. The amino acid content revealed no unusual features, but analysis by the method of Barrantes (F. Barrantes, Biochem. Biophys. Res. Commun. 62:407-414, 1975) indicated it is likely a peripheral membrane protein. The protein makes up approximately 1% of the total cell protein and is a prominent surface structure. Because glycoproteins have been implicated in cellular interactions in a number of systems, the VGP may play an

important role in the social behavior exhibited by M. xanthus.

Mahne, M., A. Tauch, et al. (2006). "The Corynebacterium glutamicum gene pmt encoding a glycosyltransferase related to eukaryotic protein-O-mannosyltransferases is essential for glycosylation of the resuscitation promoting factor (Rpf2) and other secreted proteins." FEMS Microbiol Lett **259**(2): 226-33.
Two-dimensional gel electrophoresis and immunoassays revealed several proteins of the secretory subproteome of Corynebacterium glutamicum to be glycosylated. By genome-wide searches for genes involved in glycosylation, the C. glutamicum gene cg1014 was found to exhibit significant similarity to eukaryotic protein-O-mannosyltransferases (PMTs) and to a recently identified orthologue of Mycobacterium tuberculosis, Rv1002c, which is responsible for protein-O-mannosylation. The putative membrane protein Cg1014 showed the same predicted transmembrane topology as Saccharomyces cerevisiae PMT1 and M. tuberculosis Rv1002c along with conserved amino acid residues responsible for catalytic activity. Deletion of the C. glutamicum pmt gene (cg1014) caused a complete loss of glycosylation of secreted proteins including the resuscitation promoting factor 2 (Rpf2), which is involved in intercellular communication and growth stimulation of C. glutamicum. Because the gene pmt as well as rpf genes are present in the genomes of all actinobacteria sequenced so far, this work provides new insights into bacterial protein glycosylation and new opportunities to elucidate the molecular mechanisms of Rpf activity in pathogenic growth and infection.

Matz, L. L., T. C. Beaman, et al. (1970). "Chemical composition of exosporium from spores of Bacillus cereus." J Bacteriol **101**(1): 196-201.
Homogeneous fragments of exosporium were extricated in centigram amounts from dormant spores of Bacillus cereus and analyzed for intrinsic constituents. The membrane proved to be chemically complex but not unique, consisting mainly of protein (52%), amino and neutral polysaccharides (20%), lipids (18%), and ash (4%). Seventeen common amino acids were identified by chromatography, and were present in usual proportions except for low levels of cystine-cysteine, methionine, tyrosine, and histidine. Glucosamine was the only amino sugar, and glucose and rhamnose were the principal neutral sugars. The lipid fraction contained 5.5% cardiolipin as the only phospholipid, 12.5% neutral lipids, and at least 19 fatty acids, among which normal C(16) and C(18) ones predominated. Calcium and phosphorus occurred in the ash. Small amounts of teichoic, ribonucleic, and dipicolinic acids were believed to represent contamination.

Mauri, P. L., P. G. Pietta, et al. (1999). "Characterization of surface layer proteins from Clostridium difficile by liquid chromatography/electrospray ionization mass spectrometry." Rapid Commun Mass Spectrom **13**(8): 695-703.
Surface layers (S-layers) are regularly ordered protein subunits found as the outermost cell envelope component of many bacteria. Most S-layers are composed of a single protein or glycoprotein species with a molecular weight

varying between 40 and 200 kDa. Clostridium difficile is the most common cause of antibiotic associated diarrhea (AAD) and pseudomembranous colitis (PMC) in humans. Detection of the S-layer in some C. difficile strains, and preliminary characterization of two glycoproteins, P36 and P47, involved in the composition of the S-layer of one of these strains (C. difficile C253), led us to investigate the most appropriate conditions for purification and chemical characterization of these proteins. This work describes the results obtained when liquid chromatography (LC) coupled to mass spectrometry (MS) using electrospray ionization was applied to the analysis of C. difficile S-layer proteins (SLPs). In this way the molecular weights of the two SLP components, P36 and P47, were detected to be 34,258 +/- 2 and 39,545 +/- 3 Da, respectively. These data deviate from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) results by 1.85 and 7.5 kDa. To confirm the LC-MS results, an alternative molecular weight analysis was performed: the two S-layer proteins were isolated by semipreparative high performance liquid chromatography (HPLC), concentrated, and analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). The two SLP subunits were digested with protease V8, and the peptide maps were determined by LC-MS using a C18 column. Finally, preliminary results about peptide glycosylation were obtained.

McBride, J. W., X. J. Yu, et al. (2000). "Glycosylation of homologous immunodominant proteins of Ehrlichia chaffeensis and Ehrlichia canis." Infect Immun **68**(1): 13-8.

The glycoprotein genes of Ehrlichia chaffeensis (1,644 bp) and Ehrlichia canis (2,064 bp) encode proteins of 548 to 688 amino acids with predicted molecular masses of only 61 and 73 kDa but with electrophoretic mobilities of 120 kDa (P120) and 140 kDa (P140), respectively. The 120-kDa protein gene of E. chaffeensis contains four identical 240-bp tandem repeat units, and the 140-kDa protein gene of E. canis has 14 nearly identical, tandemly arranged 108-bp repeat units. Conserved serine-rich motifs identified in the repeat units of P120 and P140 were also found in the repeat units of the human granulocytotropic ehrlichiosis agent 130-kDa protein and of the fimbria-associated adhesin protein Fap1 of Streptococcus parasanguis. Nearly the entire (99%) E. chaffeensis P120 gene (1,616 bp), the 14-repeat region (78%) of the E. canis P140 gene (1,620 bp), and a 2-repeat region from the E. chaffeensis P120 gene (520 bp) were expressed in Escherichia coli. The recombinant proteins exhibited molecular masses ranging from 1.6 to 2 times larger than those predicted by the amino acid sequences. Antibodies against the recombinant proteins reacted with E. chaffeensis P120 and E. canis P140, respectively. Carbohydrate was detected on the E. chaffeensis and E. canis recombinant proteins, including the two-repeat polypeptide region of E. chaffeensis P120. A carbohydrate compositional analysis identified glucose, galactose, and xylose on the recombinant proteins. The presence of only one site for N-linked (Asn-Xaa-Ser/Thr) glycosylation, a lack of effect of N-glycosidase F, the presence of 70 and 126 Ser/Thr glycosylation sites in the repeat regions of P120 and P140, respectively, and a high molar ratio of carbohydrate to protein suggest that the glycans may be O linked.

McManus, J. D., D. C. Brune, et al. (1992). "Isolation, characterization, and amino acid sequences of auracyanins, blue copper proteins from the green photosynthetic bacterium Chloroflexus aurantiacus." J Biol Chem **267**(10): 6531-40.

Three small blue copper proteins designated auracyanin A, auracyanin B-1, and auracyanin B-2 have been isolated from the thermophilic green gliding photosynthetic bacterium Chloroflexus aurantiacus. All three auracyanins are peripheral membrane proteins. Auracyanin A was described previously (Trost, J. T., McManus, J. D., Freeman, J. C., Ramakrishna, B. L., and Blankenship, R. E. (1988) Biochemistry 27, 7858-7863) and is not glycosylated. The two B forms are glycoproteins and have almost identical properties to each other, but are distinct from the A form. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis apparent monomer molecular masses are 14 (A), 18 (B-2), and 22 (B-1) kDa. The amino acid sequences of the B forms are presented. All three proteins have similar absorbance, circular dichroism, and resonance Raman spectra, but the electron spin resonance signals are quite different. Laser flash photolysis kinetic analysis of the reactions of the three forms of auracyanin with lumiflavin and flavin mononucleotide semiquinones indicates that the site of electron transfer is negatively charged and has an accessibility similar to that found in other blue copper proteins. Copper analysis indicates that all three proteins contain 1 mol of copper per mol of protein. All three auracyanins exhibit a midpoint redox potential of +240 mV. Light-induced absorbance changes and electron spin resonance signals suggest that auracyanin A may play a role in photosynthetic electron transfer. Kinetic data indicate that all three proteins can donate electrons to cytochrome c-554, the electron donor to the photosynthetic reaction center.

Meier, B., C. M. Brunotte, et al. (1992). "Isolation of a high-molecular mass glycoprotein from culture supernatant of an arthritogenic strain of the bacteria Erysipelothrix rhusiopathiae reacting with "inductive" monoclonal antibodies derived from rats with erysipelas polyarthritis." Biol Chem Hoppe Seyler **373**(8): 715-21.

A glycoprotein exhibiting a relative molecular mass of about 1000 kDa was purified to homogeneity from culture supernatant of arthritogenic bacteria (Erysipelothrix rhusiopathiae, strain T28) by ultrafiltration, ammonium sulfate precipitation, molecular mass exclusion, and ion exchange chromatography. Fractions obtained were analysed for their antigenic content by an enzyme linked immunosorbent assay (ELISA) using rabbit immune serum raised against this strain of Erysipelothrix rhusiopathiae. Distinct monoclonal antibodies obtained from rats suffering from erysipelas polyarthritis display a unique property by inducing very efficiently protective and regulatory mechanisms while being unable to generate classical "passive immunity". These "inductive" monoclonal antibodies recognize most likely linear epitopes on the purified glycoprotein. This makes it a prime source for analysing the target structure of these in vivo "inductive" antibodies.

Meldgaard, M. and I. Svendsen (1994). "Different effects of N-glycosylation on the thermostability of highly homologous bacterial (1,3-1,4)-beta-glucanases secreted from

yeast." Microbiology **140 (Pt 1)**: 159-66.

Genes encoding Bacillus amyloliquefaciens (1,3-1,4)-beta-glucanase (AMY), B. macerans (1,3-1,4)-beta-glucanase (MAC), and a series of hybrid enzymes containing N-terminal sequence segments of different length derived from AMY with the remaining C-terminal segment derived from MAC, were expressed in Saccharomyces cerevisiae. The cells secreted active enzyme into the medium. While the quantity of N-glycan linked to the different enzymes was similar, pronounced differences in thermotolerance were observed when the glycosylated enzymes were compared with the unglycosylated counterparts produced in Escherichia coli. Glycosylated AMY and hybrid enzyme H(A16-M), consisting of 16 N-terminal amino acids derived from AMY with the remaining C-terminal segment from MAC, exhibited a 7.5- and 1.6-fold increase in half-life at 70 degrees C, pH 6.0. N-terminal sequencing established that only two out of three sites for potential N-glycosylation of H(A16-M) secreted from yeast were actually glycosylated. Removal of N-glycans by endoglycosidase H and peptide:N-glycosidase F from H(A16-M) resulted in a 16- and 133-fold decrease of thermostability, demonstrating that N-glycans are a major determinant for the resistance of this enzyme to thermal inactivation. Glycosylated MAC and hybrid enzymes H(A36-M), H(A107-M) and H(A152-M) had increased thermostability but hybrid enzyme H(A78-M) was less thermostable. N-Glycosylation thus changes thermostability of (1,3-1,4)-beta-glucanases with similar primary structure in a variable, so far unpredictable way.

Menozzi, F. D., R. Bischoff, et al. (1998). "Molecular characterization of the mycobacterial heparin-binding hemagglutinin, a mycobacterial adhesin." Proc Natl Acad Sci U S A **95**(21): 12625-30.

Although it generally is accepted that the interaction of Mycobacterium tuberculosis with alveolar macrophages is a key step in the pathogenesis of tuberculosis, interactions with other cell types, especially epithelial cells, also may be important. In this study we describe the molecular characterization of a mycobacterial heparin-binding hemagglutinin (HBHA), a protein that functions as an adhesin for epithelial cells. The structural gene was cloned from M. tuberculosis and bacillus Calmette-Guerin, and the sequence was found to be identical between the two species. The calculated Mr was smaller than the observed Mr when analyzed by SDS/PAGE. This difference can be attributed to the Lys/Pro-rich repeats that occur at the C-terminal end of the protein and to a putative carbohydrate moiety. Glycosylation of HBHA appears to protect the protein from proteolytic degradation, which results in the removal of the C-terminal Lys/Pro-rich region responsible for binding of HBHA to sulfated carbohydrates. Evidence suggests that glycosylation is also important for HBHA-mediated hemagglutination and for certain immunologic properties of the protein. Finally, the absence of a signal peptide in the coding region of HBHA raises the possibility that this protein is not secreted via the general secretion pathway.

Messner, P. (1997). "Bacterial glycoproteins." Glycoconj J **14**(1): 3-11.

Glycoproteins are a diverse group of complex macromolecules that are present

in virtually all forms of life. Their presence in prokaryotes, however, has been demonstrated, and accepted, only recently. Bacterial glycoproteins have been identified in many archaeobacteria and in eubacteria. They comprise a wide range of different cell envelope components such as membrane-associated glycoproteins, surface-associated glycoproteins and crystalline surface layers (S-layers), as well as secreted glycoproteins and exoenzymes. Even their occurrence in the cytoplasm cannot yet be ruled out. This minireview tries to cover the whole subject as completely as possible and refers to available information on presence, structure, biosynthesis, and molecular biology of bacterial glycoproteins.

Messner, P., K. Bock, et al. (1990). "Characterization of the surface layer glycoprotein of Clostridium symbiosum HB25." J Bacteriol **172**(5): 2576-83.

The cell surface of Clostridium symbiosum HB25 is covered by a squarely arranged surface layer (S-layer) glycoprotein. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the sodium dodecyl sulfate-soluble whole-cell extract showed the presence of several high-molecular-weight protein bands in a narrow range (approximate Mr, 140,000) which, upon periodic acid-Schiff staining, gave a positive reaction. After proteolytic degradation of the purified S-layer glycoprotein, a single glycopeptide fraction was obtained by gel permeation chromatography. Hydrolysis, treatment with aqueous hydrofluoric acid, and 1H and 13C nuclear magnetic resonance studies showed that the glycoprotein glycan is a high-molecular-weight polymer (approximate Mr, 15,000) of tetrasaccharide repeating units with the component sugars N-acetylgalactosamine (GalNAc), N-acetylmannosamine (ManNAc), and N-acetylbacillosamine (BacNAc; 2-N-acetyl-4-amino-2,4,6-trideoxy glucose) linked by monophosphate diesters. The following structure is proposed: [----6)-alpha-D-ManpNAc-(1----4)-beta-D-GalpNAc-(1----3)-alpha-D-+ ++BacpNAc- (1----4)-alpha-D-GalpNAc-(1----PO3)----]n. The nuclear magnetic resonance data provided evidence for a charge interaction between the free amino group of BacNAc and the phosphate group of adjacent glycan chains. Since polycationic ferritin did not label the cell surface of intact cells, an electrostatic interaction can also be expected in vivo, leading to a charge-neutral outer surface, which is characteristic of all other S layers from members of the family Bacillaceae studied so far.

Messner, P., R. Christian, et al. (1992). "Analysis of a novel linkage unit of O-linked carbohydrates from the crystalline surface layer glycoprotein of Clostridium thermohydrosulfuricum S102-70." J Bacteriol **174**(7): 2236-40.

The surface layer glycoprotein of Clostridium thermohydrosulfuricum S102-70 was shown to contain a new type of glycan chain. Different from all known eubacterial glycoproteins, the saccharide moiety consists only of six sugar residues without any repeat sequences. Proteolytic digestion of purified S-layer glycoprotein resulted in isolation of several glycopeptide fractions. These are composed of the same hexasaccharide portion but are linked to oligopeptides of different length. One of them contains only a single amino acid. As concluded

from chemical analyses and proton and carbon nuclear magnetic resonance spectroscopy of this preparation, the hexasaccharide moiety is linked via a novel O-glycosidic linkage. This is a beta-D-glucose residue linked to the phenolic hydroxyl group of tyrosine in intact S-layer glycoprotein.

Messner, P., R. Christian, et al. (1995). "Similarity of "core" structures in two different glycans of tyrosine-linked eubacterial S-layer glycoproteins." J Bacteriol **177**(8): 2188-93.

Previously, the repeating-unit structure of the S-layer glycoprotein from the eubacterium Bacillus alvei CCM 2051 has been determined to be [-->3)-beta-D-Galp-(1-->4)-[alpha-D-Glcp-(1-->6)-]-beta-D-ManpNAc- (1-->]n (E. Altman, J.-R. Brisson, P. Messner, and U. B. Sleytr, Biochem. Cell Biol. 69:72-78, 1991). Nuclear magnetic resonance spectroscopic reexamination of this glycan reveals that the O-antigen-like domain of the polysaccharide is [see text] connected with the S-layer polypeptide through the "core" structure -->3)-alpha-L-Rhap-(1-->3)-alpha-L-Rhap-(1-->3)-alpha-L-R hap-(1-->3)-beta-D-Galp-(1-->O)-Tyr. Except for the substitution in position 4 of the nonreducing rhamnose with the modified glyceric acid phosphate residue GroA-2-->OPO2-->4-beta-D-ManpNAc-(1-->, this core is identical to the core of the tyrosine-linked glycan from the S-layer glycoprotein of Thermoanaerobacter thermohydrosulfuricus L111-69 (K. Bock, J. Schuster-Kolbe, E. Altman, G. Allmaier, B. Stahl, R. Christian, U. B. Sleytr, and P. Messner, J. Biol. Chem. 269:7137-7144, 1994).

Messner, P. and C. Schaffer (2003). "Prokaryotic glycoproteins." Fortschr Chem Org Naturst **85**: 51-124.

Messner, P., K. Steiner, et al. (2008). "S-layer nanoglycobiology of bacteria." Carbohydr Res **343**(12): 1934-51.

Cell surface layers (S-layers) are common structures of the bacterial cell envelope with a lattice-like appearance that are formed by a self-assembly process. Frequently, the constituting S-layer proteins are modified with covalently linked glycan chains facing the extracellular environment. S-layer glycoproteins from organisms of the Bacillaceae family possess long, O-glycosidically linked glycans that are composed of a great variety of sugar constituents. The observed variations already exceed the display found in eukaryotic glycoproteins. Recent investigations of the S-layer protein glycosylation process at the molecular level, which has lagged behind the structural studies due to the lack of suitable molecular tools, indicated that the S-layer glycoprotein glycan biosynthesis pathway utilizes different modules of the well-known biosynthesis routes of lipopolysaccharide O-antigens. The genetic information for S-layer glycan biosynthesis is usually present in S-layer glycosylation (slg) gene clusters acting in concert with housekeeping genes. To account for the nanometer-scale cell surface display feature of bacterial S-layer glycosylation, we have coined the neologism 'nanoglycobiology'. It includes structural and biochemical aspects of S-layer glycans as well as molecular data on the machinery underlying the glycosylation event. A key aspect for the full potency of S-layer nanoglycobiology

is the unique self-assembly feature of the S-layer protein matrix. Being aware that in many cases the glycan structures associated with a protein are the key to protein function, S-layer protein glycosylation will add a new and valuable component to an 'S-layer based molecular construction kit'. In our long-term research strategy, S-layer nanoglycobiology shall converge with other functional glycosylation systems to produce 'functional' S-layer neoglycoproteins for diverse applications in the fields of nanobiotechnology and vaccine technology. Recent advances in the field of S-layer nanoglycobiology have made our overall strategy a tangible aim of the near future.

Morris, E. J., N. Ganeshkumar, et al. (1987). "Identification and preliminary characterization of a Streptococcus sanguis fibrillar glycoprotein." J Bacteriol **169**(1): 164-71.

Cell surface fibrils could be released from Streptococcus sanguis 12 but not from strains 12na or N by freeze-thawing followed by brief homogenization. Fibrils were isolated from the homogenate by ultracentrifugation or ammonium sulfate precipitation. Electron microscopy demonstrated the presence of dense masses of aggregated fibrils in these preparations. Under nondenaturing conditions, no proteins were seen in polyacrylamide gel electrophoresis (PAGE). Sodium dodecyl sulfate (SDS)-PAGE analysis revealed a single band stained with Coomassie blue and periodic acid Schiff stain with a molecular weight in excess of 300,000. The protein has been given the name long-fibril protein (LFP). The molecule was susceptible to digestion with subtilisin, pronase, papain, and trypsin, but was unaffected by chymotrypsin or muramidases. Attempts to dissociate the protein into smaller subunits with urea, guanidine, sodium thiocyanate, and HCl were unsuccessful. Gel filtration on a column of Sephacryl S-400 in the presence of 2% SDS resulted in elution of the protein at the void volume. Antibody raised against the LFP excised from an SDS-PAGE gel reacted with long fibrils on the surface of strain 12 and with isolated fibrils by an immunogold labeling technique. Monoclonal antibody reactive with LFP in SDS-PAGE also reacted with fibrils present on the cell. Antisera raised against the fibrils inhibited adherence to saliva-coated hydroxyapatite.

Moskophidis, M. and F. Muller (1984). "Molecular characterization of glycoprotein antigens on surface of Treponema pallidum: comparison with nonpathogenic Treponema phagedenis biotype Reiter." Infect Immun **46**(3): 867-9.

Four glycoproteins of Treponema pallidum were identified by intrinsic [14C]glucosamine labeling. Only two glycoproteins were demonstrated in T. phagedenis biotype Reiter with the same technique. Glycoproteins of both treponemes were characterized as antigens and shown to be localized within the outer membranes of the microorganisms.

Moskophidis, M. and F. Muller (1985). "Identification of glycosylated protein antigens of Treponema pallidum and Treponema phagedenis." Zentralbl Bakteriol Mikrobiol Hyg A **259**(4): 468-76.

Comparison of autoradiographies of intrinsically [35S] methionine and [14C]

glucosamine labeled Treponema pallidum (Nichols strain) after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed four glycosylated proteins with molecular weights 30,500, 33,000, 35,000, and 59,000. T. phagedenis (biotype Reiter) was comparatively investigated and showed only two glycosylated proteins with molecular weights 33,000 and 34,000. The at the first time in treponemes identified glycosylated proteins could be precipitated with homologous human antibodies and characterized as antigens. By comparison with 125I surface labeling of T. pallidum and T. phagedenis it is suggested that the glycosylated protein antigens are localized on the surface of these treponemes.

Oberli, M. A., M. Tamborrini, et al. "Molecular analysis of carbohydrate-antibody interactions: case study using a Bacillus anthracis tetrasaccharide." J Am Chem Soc **132**(30): 10239-41.

The process for selecting potent and effective carbohydrate antigens is not well-established. A combination of synthetic glycan microarray screening, surface plasmon resonance analysis, and saturation transfer difference NMR spectroscopy was used to dissect the antibody-binding surface of a carbohydrate antigen, revealing crucial binding elements with atomic-level detail. This analysis takes the first step toward uncovering the rules for structure-based design of carbohydrate antigens.

Okuda, S. and G. Weinbaum (1968). "An envelope-specific glycoprotein from Escherichia coli B." Biochemistry **7**(8): 2819-25.

Ong, E., D. G. Kilburn, et al. (1994). "Streptomyces lividans glycosylates the linker region of a beta-1,4-glycanase from Cellulomonas fimi." J Bacteriol **176**(4): 999-1008.

The beta-1,4-glycanase Cex of the gram-positive bacterium Cellulomonas fimi is a glycoprotein comprising a C-terminal cellulose-binding domain connected to an N-terminal catalytic domain by a linker containing only prolyl and threonyl (PT) residues. Cex is also glycosylated by Streptomyces lividans. The glycosylation of Cex produced in both C. fimi and S. lividans protects the enzyme from proteolysis. When the gene fragments encoding the cellulose-binding domain of Cex (CBDCex), the PT linker plus CBDCex (PT-CBDCex), and the catalytic domain plus CBDCex of Cex were expressed in S. lividans, only PT-CBDCex was glycosylated. Therefore, all the glycans must be O linked because only the PT linker was glycosylated. A glycosylated form and a nonglycosylated form of PT-CBDCex were produced by S. lividans. The glycosylated form of PT-CBDCex was heterogeneous; its average carbohydrate content was approximately 10 mol of D-mannose equivalents per mol of protein, but the glycans contained from 4 to 12 alpha-D-mannosyl and alpha-D-galactosyl residues. Glycosylated Cex from S. lividans was also heterogeneous. The presence of glycans on PT-CBDCex increased its affinity for bacterial microcrystalline cellulose. The location of glycosylation only on the linker region of Cex correlates with the properties conferred on the enzyme by the glycans.

Paramonov, N., M. Rangarajan, et al. (2005). "Structural analysis of a novel anionic polysaccharide from Porphyromonas gingivalis strain W50 related to Arg-gingipain glycans." Mol Microbiol **58**(3): 847-63.

The Arg-gingipains (RgpsA and B) of Porphyromonas gingivalis are a family of extracellular cysteine proteases and are important virulence determinants of this periodontal bacterium. A monoclonal antibody, MAb1B5, which recognizes an epitope on glycosylated monomeric RgpAs also cross-reacts with a cell-surface polysaccharide of P. gingivalis W50 suggesting that the maturation pathway of the Arg-gingipains may be linked to the biosynthesis of a surface carbohydrate. We report the purification and structural characterization of the cross-reacting anionic polysaccharide (APS), which is distinct from both the lipopolysaccharide and serotype capsule polysaccharide of P. gingivalis W50. The structure of APS was determined by 1D and 2D NMR spectroscopy and methylation analysis, which showed it to be a phosphorylated branched mannan. The backbone is built up of alpha-1,6-linked mannose residues and the side-chains contain alpha-1,2-linked mannose oligosaccharides of different lengths (one to two sugar residues) attached to the backbone via 1,2-linkage. One of the side-chains in the repeating unit contains Manalpha1-2Manalpha1-phosphate linked via phosphorus to a backbone mannose at position 2. De-O-phosphorylation of APS abolished cross-reactivity suggesting that Manalpha1-2Manalpha1-phosphate fragment forms part of the epitope recognized by MAb1B5. This phosphorylated branched mannan represents a novel polysaccharide that is immunologically related to the post-translational additions of Arg-gingipains.

Peters, J., M. Nitsch, et al. (1995). "Tetrabrachion: a filamentous archaebacterial surface protein assembly of unusual structure and extreme stability." J Mol Biol **245**(4): 385-401.

The surface (S-) layer of the hyperthermophilic archaebacterium Staphylothermus marinus was isolated, dissected into separate domains by chemical and proteolytic methods, and analyzed by spectroscopic, electron microscopic and biochemical techniques. The S-layer is formed by a poorly ordered meshwork of branched, filiform morphological subunits resembling dandelion seed-heads. A morphological subunit (christened by us tetrabrachion) consists of a 70 nm long, almost perfectly straight stalk ending in four straight arms of 24 nm length that provide lateral connectivity by end-to-end contacts. At 32 nm from the branching point, tetrabrachion carries two globular particles of 10 nm diameter that have both tryptic and chymotryptic protease activity. Tetrabrachion is built by a tetramer of M(r) 92,000 polypeptides that form a parallel, four-stranded alpha-helical rod and separate at one end into four strands. These strands interact in a 1:1 stoichiometry with polypeptides of M(r) 85,000 to form the arms. The arms are composed entirely of beta-sheets. All S-layer components contain bound carbohydrates (glucose, mannose, and glucosamine) at a ratio of 38 g/100 g protein for the complete tetrabrachion-protease complex. The unique structure of tetrabrachion is reflected in an extreme thermal stability in the presence of strong denaturants (1% (w/v) SDS of 6M guanidine): the arms, which are stabilized by intramolecular disulphide

bridges, melt around 115 degrees C under non-reducing conditions, whereas the stalk sustains heating up to about 130 degrees C. Complete denaturation of the stalk domain requires treatment with 70% (v/v) sulfuric acid or with fuming trifluoromethanesulfonic acid. The globular protease can be heated to 90 degrees C in 6M guanidine and to 120 degrees C in 1% SDS and represents one of the most stable proteases characterized to date.

Peters, J., M. Peters, et al. (1987). "Nucleotide sequence analysis of the gene encoding the Deinococcus radiodurans surface protein, derived amino acid sequence, and complementary protein chemical studies." J Bacteriol **169**(11): 5216-23.

The complete nucleotide sequence of the gene encoding the surface (hexagonally packed intermediate [HPI])-layer polypeptide of Deinococcus radiodurans Sark was determined and found to encode a polypeptide of 1,036 amino acids. Amino acid sequence analysis of about 30% of the residues revealed that the mature polypeptide consists of at least 978 amino acids. The N terminus was blocked to Edman degradation. The results of proteolytic modification of the HPI layer in situ and Mr estimations of the HPI polypeptide expressed in Escherichia coli indicated that there is a leader sequence. The N-terminal region contained a very high percentage (29%) of threonine and serine, including a cluster of nine consecutive serine or threonine residues, whereas a stretch near the C terminus was extremely rich in aromatic amino acids (29%). The protein contained at least two disulfide bridges, as well as tightly bound reducing sugars and fatty acids.

Pethe, K., V. Puech, et al. (2001). "Mycobacterium smegmatis laminin-binding glycoprotein shares epitopes with Mycobacterium tuberculosis heparin-binding haemagglutinin." Mol Microbiol **39**(1): 89-99.

Mycobacterium tuberculosis, the causative agent of tuberculosis, produces a heparin-binding haemagglutinin adhesin (HBHA), which is involved in its epithelial adherence. To ascertain whether HBHA is also present in fast-growing mycobacteria, Mycobacterium smegmatis was studied using anti-HBHA monoclonal antibodies (mAbs). A cross-reactive protein was detected by immunoblotting of M. smegmatis whole-cell lysates. However, the M. tuberculosis HBHA-encoding gene failed to hybridize with M. smegmatis chromosomal DNA in Southern blot analyses. The M. smegmatis protein recognized by the anti-HBHA mAbs was purified by heparin-Sepharose chromatography, and its amino-terminal sequence was found to be identical to that of the previously described histone-like protein, indicating that M. smegmatis does not produce HBHA. Biochemical analysis of the M. smegmatis histone-like protein shows that it is glycosylated like HBHA. Immunoelectron microscopy demonstrated that the M. smegmatis protein is present on the mycobacterial surface, a cellular localization inconsistent with a histone-like function, but compatible with an adhesin activity. In vitro protein interaction assays showed that this glycoprotein binds to laminin, a major component of basement membranes. Therefore, the protein was called M. smegmatis laminin-binding protein (MS-LBP). MS-LBP does not appear to be involved in adherence in the absence of laminin but is responsible for the

laminin-mediated mycobacterial adherence to human pneumocytes and macrophages. Homologous laminin-binding adhesins are also produced by virulent mycobacteria such as M. tuberculosis and Mycobacterium leprae, suggesting that this adherence mechanism may contribute to the pathogenesis of mycobacterial diseases.

Peyret, J. L., N. Bayan, et al. (1993). "Characterization of the cspB gene encoding PS2, an ordered surface-layer protein in Corynebacterium glutamicum." Mol Microbiol **9**(1): 97-109.

PS2 is one of two major proteins detected in the culture media of various Corynebacterium glutamicum strains. The coding and promoter regions of the cspB gene encoding PS2 were cloned in lambda gt11 using polyclonal antibodies raised against PS2 for screening. Expression of the cspB gene in Escherichia coli led to the production of a major anti-PS2 labelled peptide of 63,000 Da, corresponding presumably to the mature form of PS2. It was detected in the cytoplasm, periplasm and surrounding medium of E. coli. Three other slower migrating bands of 65,000 68,000 and 72,000 Da were detected. The largest one probably corresponds to the precursor form of PS2 in E. coli. Analysis of the nucleotide sequence revealed an open reading frame (ORF) of 1533 nucleotides. The deduced 510-amino-acid polypeptide had a calculated molecular mass of 55,426 Da. According to the predicted amino acid sequence, PS2 is synthesized with a N-terminal segment of 30-amino-acid residues reminiscent of eukaryotic and prokaryotic signal peptides, and a hydrophobic domain of 21 residues near the C-terminus. Although no significant homologies were found with other proteins, it appears that some characteristics and the amino acid composition of PS2 share several common features with surface-layer proteins. The cspB gene was then disrupted in C. glutamicum by gene replacement. Freeze-etching electron microscopy performed on the wild-type strain indicated that the cell wall of C. glutamicum is covered with an ordered surface of proteins (surface layer, S-layer) which is in very close contact with other cell-wall components. These structures are absent from the cspB-disrupted strain but are present after reintroduction of the cspB gene on a plasmid into this mutant. Thus we demonstrate that the S-layer protein is the product of the cspB gene.

Plummer, C., H. Wu, et al. (2005). "A serine-rich glycoprotein of Streptococcus sanguis mediates adhesion to platelets via GPIb." Br J Haematol **129**(1): 101-9.

Streptococcus sanguis is the most common oral bacterium causing infective endocarditis and its ability to adhere to platelets, leading to their activation and aggregation, is thought to be an important virulent factor. Previous work has shown that S. sanguis can bind directly to platelet glycoprotein (GP) Ib but the nature of the adhesin was unknown. Here, we have shown that a high molecular weight glycoprotein of S. sanguis mediates adhesion to glycocalacin. The bacterial glycoprotein was purified from cell extracts by chromatography on GPIb- and wheatgerm agglutinin affinity matrices and its interaction with GPIb was shown to be sialic acid-dependent. We designated the glycoprotein serine-rich protein A (SrpA). An insertional inactivation mutant lacking the SrpA of S.

sanguis showed significantly reduced binding to glycocalacin, reduced adherence to platelets and a prolonged lag time to platelet aggregation. In addition, under flow conditions, platelets rolled and subsequently adhered on films of wild-type S. sanguis cells at low shear (50/s) but did not bind to films of the SrpA mutant. Platelets did not bind to wild-type bacterial cells at high shear (1500/s). These findings help to understand the mechanisms by which the organism might colonize platelet-fibrin vegetations.

Porstendorfer, D., O. Gohl, et al. (2000). "ComP, a pilin-like protein essential for natural competence in Acinetobacter sp. Strain BD413: regulation, modification, and cellular localization." J Bacteriol **182**(13): 3673-80.

We recently identified a pilin-like competence factor, ComP, which is essential for natural transformation of the gram-negative soil bacterium Acinetobacter sp. strain BD413. Here we demonstrate that transcription and synthesis of the pilin-like competence factor ComP are maximal in the late stationary growth phase, whereas competence is induced immediately after inoculation of a stationary-phase culture into fresh medium. Western blot analyses revealed three forms of ComP, one with an apparent molecular mass of 15 kDa, which correlates with the molecular mass deduced from the DNA sequence, one 20-kDa form, which was found to be glycosylated, and one 23-kDa form. The glycosylation of ComP was not required for its function in DNA binding and uptake. The 20-kDa form was present in the cytoplasmic membrane, the periplasm, and the outer membrane, whereas the 23-kDa form was located in the outer membrane and might be due to a further modification. Immunological data suggest that ComP is not a subunit of the pilus structures. Possible functions of ComP in the DNA transformation machinery of Acinetobacter sp. strain BD413 are discussed.

Rangarajan, M., A. Hashim, et al. (2005). "Expression of Arg-Gingipain RgpB is required for correct glycosylation and stability of monomeric Arg-gingipain RgpA from Porphyromonas gingivalis W50." Infect Immun **73**(8): 4864-78.

Arg-gingipains are extracellular cysteine proteases produced by the gram-negative periodontal pathogen Porphyromonas gingivalis and are encoded by rgpA and rgpB. Three Arg-gingipains, heterodimeric high-molecular-mass Arg-gingipain HRgpA comprising the alpha-catalytic chain and the beta-adhesin chain, the monomeric soluble Arg-gingipain comprising only the alpha-catalytic chain (RgpA(cat)), and the monomeric membrane-type heavily glycosylated Arg-gingipain comprising the alpha-catalytic chain (mt-RgPA(cat)), are derived from rgpA. The monomeric enzymes contain between 14 and 30% carbohydrate by weight. rgpB encodes two monomeric enzymes, RgpB and mt-RgpB. Earlier work indicated that rgpB is involved in the glycosylation process, since inactivation of rgpB results in the loss of not only RgpB and mt-RgpB but also mt-RgpA(cat). This work aims to confirm the role of RgpB in the posttranslational modification of RgpA(cat) and the effect of aberrant glycosylation on the properties of this enzyme. Two-dimensional gel electrophoresis of cellular proteins from W50 and an inactivated rgpB strain (D7) showed few differences, suggesting that loss of RgpB has a specific effect on RgpA maturation.

Inactivation of genes immediately upstream and downstream of rgpB had no effect on rgpA-derived enzymes, suggesting that the phenotype of the rgpB mutant is not due to a polar effect on transcription at this locus. Matrix-assisted laser desorption ionization-time of flight analysis of purified RgpA(cat) from W50 and D7 strains gave identical peptide mass fingerprints, suggesting that they have identical polypeptide chains. However, RgpA(cat) from D7 strain had a higher isoelectric point and a dramatic decrease in thermostability and did not cross-react with a monoclonal antibody which recognizes a glycan epitope on the parent strain enzyme. Although it had the same total sugar content as the parent strain enzyme, there were significant differences in the monosaccharide composition and linking sugars. These data suggest that RgpB is required for the normal posttranslational glycosylation of Arg-gingipains derived from rgpA and that this process is required for enzyme stabilization.

Rangarajan, M., S. J. Smith, et al. (1997). "Biochemical characterization of the arginine-specific proteases of Porphyromonas gingivalis W50 suggests a common precursor." Biochem J **323 (Pt 3)**: 701-9.

Extracellular proteases of Porphyromonas gingivalis specific for arginyl peptide bonds are considered to be important virulence factors in periodontal disease. In order to determine the number, inter-relationship and kinetic properties of these proteases, extracellular enzymes with this peptide-bond specificity were purified and characterized from P. gingivalis W50. Three forms, which we denote RI, RI-A and RI-B, accounted for all of the activity in the supernatant. All three enzymes contain an alpha chain of approximately 54 kDa with the same N-terminal amino acid sequence. RI is a heterodimer of non-covalently linked alpha and beta chains which migrate to the same position on SDS/PAGE but which can be resolved by 8 M urea/PAGE. RI-A and RI-B are both monomeric, but the molecular mass of RI-B (70-80 kDa) is significantly increased due to post-translational modification with lipopolysaccharide. All forms show absolute specificity for peptide bonds with Arg in the P1 position and are also capable of hydrolysing N-terminal Arg and C-terminal Arg-Arg peptide bonds. Thus they show limited amino- and carboxy-peptidase activity. For the hydrolysis of Nalpha-benzoyl-L-Arg-p-nitroanilide, the pH optimum is 8.0 at 30 degrees C. The Vmax for all three enzymes is controlled by ionization of two residues with apparent pKas at 30 degrees C of 6. 5+/-0.05 and 9.7+/-0.05, and DeltaH values of approximately 29 kJ/mol and approximately 24 kJ/mol in the enzyme-substrate complex. By analogy with papain, the pKa of 6.5 could be ascribed to a Cys and the pKa of 9.7 to a His residue. E-64 [L-trans-epoxysuccinyl-leucylamide-4-(4-guanidino)butane] is a competitive inhibitor of RI, RI-A and RI-B. Based on physical properties and kinetic behaviour, RI-A appears to be analogous to gingipain from P. gingivalis HG66. However the alpha/beta structure of RI differs significantly from that of the high-molecular-mass multimeric complex of gingipain containing four haemagglutinins described by others. Since the genes for RI and high-molecular-mass gingipain are identical, the data indicate that an alternative processing pathway is involved in the formation of RI from the initial precursor. Furthermore, the identical N-termini and enzymic properties of the catalytic

component of RI, RI-A and RI-B suggest that the maturation pathway of the RI precursor may also give rise to RI-A and RI-B. The physiological functions of these isoforms and their role in the disease process may become more apparent through examination of their interactions with host proteins.

Recht, J. and R. Kolter (2001). "Glycopeptidolipid acetylation affects sliding motility and biofilm formation in Mycobacterium smegmatis." J Bacteriol **183**(19): 5718-24.
The absence of glycopeptidolipids (GPLs) abolishes the ability of mycobacteria both to slide over the surface of motility plates and to form biofilms on polyvinyl chloride. In a screen for biofilm-defective mutants of Mycobacterium smegmatis mc(2)155, a new mutant was obtained that resulted in partial inhibition of both processes and also showed an intermediate rough colony morphology. The mariner transposon insertion mapped to a GPL biosynthesis gene (atf1) which encodes a putative acetyltranferase involved in the transfer of acetyl groups to the glycopeptide core. Physical characterization of the GPLs from the atf1 mutant demonstrated that they were not acetylated.

Rosales-Borjas, D. M., S. Zambrano-Villa, et al. (1998). "Rapid screening test for tuberculosis using a 38-kDa antigen from Mycobacterium tuberculosis." J Clin Lab Anal **12**(2): 126-9.
A screening test for the diagnosis of tuberculosis by immunodot (IDt) is described, using an antigen of Mycobacterium tuberculosis, namely, a 38-kDa glycoprotein which has shown great specificity in previous serologic analyses. The test was used to examine 28 sera from patients with lung tuberculosis. Of these, 85% were positive by micro-ELISA and by the IDt test herein described. Control sera from healthy subjects (n = 20) gave negative results for ELISA and for IDt, which indicates that the screening test is highly specific. The test is easy to handle and requires no equipment and is therefore particularly useful for field studies.

Roy, K., D. Hamilton, et al. (2009). "Vaccination with EtpA glycoprotein or flagellin protects against colonization with enterotoxigenic Escherichia coli in a murine model." Vaccine **27**(34): 4601-8.
Enterotoxigenic Escherichia coli (ETEC) remain a leading cause diarrheal illness, prompting a search for vaccine targets that led to the recent discovery of EtpA, a secreted adhesin of ETEC that acts by bridging flagella and host cells. In a murine model, immunization with recombinant EtpA glycoprotein inhibited colonization by two EtpA-producing human ETEC strains, H10407 and E24377A. In addition, vaccination with recombinant flagellin (serotype H11) generated antibodies that specifically recognized the tips of flagella from E24377A expressing a heterologous flagellar serotype (H28) and afforded significant protection against colonization. EtpA and/or flagellin could be valuable subunit antigens in the formulation of a broadly protective ETEC vaccine.

Saksena, R., R. Adamo, et al. (2006). "Synthesis of the tetrasaccharide side chain of the major glycoprotein of the Bacillus anthracis exosporium." Bioorg Med Chem Lett

16(3): 615-7.

An alpha-glycoside of the tetrasaccharide sequence beta-Ant-(1-->3)-alpha-l-Rhap-(1-->3)-alpha-l-Rhap-(1-->2)-alpha-l-Rhap whose aglycon allows conjugation to suitable carriers was synthesized. The NMR characteristics of the compound are virtually identical with those of the alpha-anomer of the tetrasaccharide isolated from the major glycoprotein of the Bacillus anthracis exosporium. Thus, the correct structure of the natural product has been proven by chemical synthesis.

Schaffer, C., K. Dietrich, et al. (2000). "A novel type of carbohydrate-protein linkage region in the tyrosine-bound S-layer glycan of Thermoanaerobacterium thermosaccharolyticum D120-70." Eur J Biochem **267**(17): 5482-92.

The surface-layer (S-layer) protein of Thermoanaerobacterium thermosaccharolyticum D120-70 contains glycosidically linked glycan chains with the repeating unit structure -->4)[alpha-D-Galp-(1-->2)]-alpha-L-Rhap-(1-->3)[beta-D-Glcp-(1--> 6)] -beta-D-Manp-(1-->4)-alpha-L-Rhap-(1-->3)-alpha-D-Glcp-(1--> . After proteolytic degradation of the S-layer glycoprotein, three glycopeptide pools were isolated, which were analyzed for their carbohydrate and amino-acid compositions. In all three pools, tyrosine was identified as the amino-acid constituent, and the carbohydrate compositions corresponded to the above structure. Native polysaccharide PAGE showed the specific heterogeneity of each pool. For examination of the carbohydrate-protein linkage region, the S-layer glycan chain was partially hydrolyzed with trifluoroacetic acid. 1D and 2D NMR spectroscopy, including a novel diffusion-edited difference experiment, showed the O-glycosidic linkage region beta-D-glucopyranose-->O-tyrosine. No evidence was found of additional sugars originating from a putative core region between the glycan repeating units and the S-layer polypeptide. For the determination of chain-length variability in the S-layer glycan, the different glycopeptide pools were investigated by matrix-assisted laser desorption ionization-time of flight mass spectrometry, revealing that the degree of polymerization of the S-layer glycan repeats varied between three and 10. All masses were assigned to multiples of the repeating units plus the peptide portion. This result implies that no core structure is present and thus supports the data from the NMR spectroscopy analyses. This is the first observation of a bacterial S-layer glycan without a core region connecting the carbohydrate moiety with the polypeptide portion.

Schirm, M., I. C. Schoenhofen, et al. (2005). "Identification of unusual bacterial glycosylation by tandem mass spectrometry analyses of intact proteins." Anal Chem **77**(23): 7774-82.

The characterization of protein glycosylation can be a complex and time-consuming procedure, especially for prokaryote O-linked glycoproteins, which often comprise unusual oligosaccharide structures with no known glycosylation motif. In this report, we describe a "top-down" approach that provides information on the extent of glycosylation, the molecular masses, and the structure of oligosaccharide residues on bacterial flagella, important structural proteins

involved in the motility of pathogenic bacteria. Flagella from four bacterial pathogens, namely, Campylobacter jejuni, Helicobacter pylori, Aeromonas caviae, and Listeria monocytogenes, were analyzed by this top-down mass spectrometry approach. The approach needs minimal sample preparation and can be performed within a few minutes compared to the tedious and often time-consuming "bottom-up" approach involving proteolytic digestion and LC-MS-MS analyses of the suspected glycopeptides. Multiply protonated protein precursor ions subjected to low-energy collisional activation in a quadrupole time-of-flight instrument showed extensive and specific gas-phase deglycosylation resulting in the formation of abundant oxonium ions with very few fragment ions from peptidic bond cleavages. Structural information on individual carbohydrate residues is obtained using a second-generation product ion scan of oxonium ions formed by collisional activation of the intact protein ions in the source region. The four bacterial flagella examined differed not only by the extent of glycosylation but also by the nature of carbohydrate substituents. For example, the flagellin from the Gram-positive bacterium, L. monocytogenes showed O-linked GlcNAc residues at up to 6 sites/protein monomer. In contrast, the three Gram-negative bacterial pathogens C. jejuni, H. pylori and A. caviae displayed up to 19 Ser/Thr O-linked sites modified with residues structurally related to N-acetylpseudaminic acid (Pse5Ac7Ac) and in the case of Campylobacter include a novel N-acetylglutamine substituent on Pse5Am7Ac.

Schirm, M., E. C. Soo, et al. (2003). "Structural, genetic and functional characterization of the flagellin glycosylation process in Helicobacter pylori." Mol Microbiol **48**(6): 1579-92.

Mass spectrometry analyses of the complex polar flagella from Helicobacter pylori demonstrated that both FlaA and FlaB proteins are post-translationally modified with pseudaminic acid (Pse5Ac7Ac, 5,7-diacetamido-3,5,7,9-tetradeoxy-l-glycero-l-manno -n o n-ulosonic acid). Unlike Campylobacter, flagellar glycosylation in Helicobacter displays little heterogeneity in isoform or glycoform distribution, although all glycosylation sites are located in the central core region of the protein monomer in a manner similar to that found in Campylobacter. Bioinformatic analysis revealed five genes (HP0840, HP0178, HP0326A, HP0326B, HP0114) homologous to other prokaryote genes previously reported to be involved in motility, flagellar glycosylation or polysaccharide biosynthesis. Insertional mutagenesis of four of these homologues in Helicobacter (HP0178, HP0326A, HP0326B, HP0114) resulted in a non-motile phenotype, no structural flagella filament and only minor amounts of flagellin protein detectable by Western immunoblot. However, mRNA levels for the flagellin structural genes remained unaffected by each mutation. In view of the combined bioinformatic and structural evidence indicating a role for these gene products in glycan biosynthesis, subsequent investigations focused on the functional characterization of the respective gene products. A novel approach was devised to identify biosynthetic sugar nucleotide precursors from intracellular metabolic pools of parent and isogenic mutants using capillary electrophoresis-electrospray mass spectrometry (CE-ESMS) and precursor ion scanning.

HP0326A, HP0326B and the HP0178 gene products are directly involved in the biosynthesis of the nucleotide-activated form of Pse, CMP-Pse. Mass spectral analyses of the cytosolic extract from the HP0326A and HP0326B isogenic mutants revealed the accumulation of a mono- and a diacetamido trideoxyhexose UDP sugar nucleotide precursor.

Schoenhofen, I. C., V. V. Lunin, et al. (2006). "Structural and functional characterization of PseC, an aminotransferase involved in the biosynthesis of pseudaminic acid, an essential flagellar modification in Helicobacter pylori." J Biol Chem **281**(13): 8907-16.

Helicobacter pylori flagellin is heavily glycosylated with the novel sialic acid-like nonulosonate, pseudaminic acid (Pse). The glycosylation process is essential for assembly of functional flagellar filaments and consequent bacterial motility. Because motility is a key virulence factor for this and other important pathogens, the Pse biosynthetic pathway offers potential for novel therapeutic targets. From recent NMR analyses, we determined that the conversion of UDP-alpha-D-Glc-NAc to the central intermediate in the pathway, UDP-4-amino-4,6-dideoxy-beta-L-AltNAc, proceeds by formation of UDP-2-acetamido-2,6-dideoxy-beta-L-arabino-4-hexulose by the dehydratase/epimerase PseB (HP0840) followed with amino transfer by the aminotransferase, PseC (HP0366). The central role of PseC in the H. pylori Pse biosynthetic pathway prompted us to determine crystal structures of the native protein, its complexes with pyridoxal phosphate alone and in combination with the UDP-4-amino-4,6-dideoxy-beta-L-AltNAc product, the latter being converted to the external aldimine form in the active site of the enzyme. In the binding site, the AltNAc sugar ring adopts a 4C1 chair conformation, which is different from the predominant 1C4 form found in solution. The enzyme forms a homodimer where each monomer contributes to the active site, and these structures have permitted the identification of key residues involved in stabilization, and possibly catalysis, of the beta-L-arabino intermediate during the amino transfer reaction. The essential role of Lys183 in the catalytic event was confirmed by site-directed mutagenesis. This work presents for the first time a nucleotide-sugar aminotransferase co-crystallized with its natural ligand, and, in conjunction with the recent functional characterization of this enzyme, these results will assist in elucidating the aminotransferase reaction mechanism within the Pse biosynthetic pathway.

Serganova, I., V. Ksenzenko, et al. (2002). "Sequencing of flagellin genes from Natrialba magadii provides new insight into evolutionary aspects of archaeal flagellins." J Bacteriol **184**(1): 318-22.

We have determined the nucleotide sequence of a flagellin gene locus from the haloalkaliphilic archaeon Natrialba magadii, identified the gene products among proteins forming flagella, and demonstrated cotranscription of the genes. Based on the sequence analysis we suggest that different regions of the genes might have distinct evolutionary histories including possible genetic exchange with bacterial flagellin genes.

Serganova, I. S., I. Polosina, et al. (1995). "[Halophilic archaea flagella: biochemical and

genetic analysis]." Biokhimiia **60**(8): 1261-7.

The protein compositions of archaebacteria (Halobacterium salinarium, Halobacterium volcanii, Halobacterium saccharovorum and Natronobacterium pharaonis 12) flagella have been studied. It was found that flagella of these archaebacterial species are made up of flagellins. The flagellins of H. salinarium, H. volcanii and H. saccharovorum are glycosylated. Based on the known primary sequences of Halobacterium halobium R1M1 flagellin genes, oligonucleotides to the 5'- and 3'-ends of locus A containing two out of five such genes have been synthesized. The amplified by primers fragment of chromosomal DNA coding for H. halobium flagellins A1 and A2 was used as a probe for detecting homologous sites in archaebacterial DNA. Southern blotting hybridization revealed that the DNA of all archaebacterial species tested in this study contains sequences that are homologous to genes flg A1 and flg A2 of H. halobium.

Shen, N. and R. M. Weiner (1998). "Isolation and characterization of S-layer proteins from a vent prosthecate bacterium." Microbios **93**(374): 7-16.

MWapp 116,000 and 29,000 proteins (p116 and p29), major outer membrane proteins of Hyphomonas jannaschiana reproductive cells, were extracted from cell envelopes by dialysis against EDTA, 2 M urea or distilled water. These proteins were precipitated by divalent cations and resolubilized by EDTA-Na, reflecting alternate monomer, multimer states. From two-dimensional gel electrophoresis it was determined that p116 and p29 had a pI of 4.5. Both were glycoproteins. Results suggest that p116 and p29 are surface layer (S-layer) proteins, with p116 a tetramer of the p29. The S-layer could protect the adherent H. jannaschiana reproductive cell from exoenzyme activity, antibiotics and other bacteriocidal molecules produced in the bacterial films formed on many marine surfaces.

Sherlock, O., U. Dobrindt, et al. (2006). "Glycosylation of the self-recognizing Escherichia coli Ag43 autotransporter protein." J Bacteriol **188**(5): 1798-807.

Glycosylation is a common modulation of protein function in eukaryotes and is biologically important. However, in bacteria protein glycosylation is rare, and relatively few bacterial glycoproteins are known. In Escherichia coli only two glycoproteins have been described to date. Here we introduce a novel member to this exclusive group, namely, antigen 43 (Ag43), a self-recognizing autotransporter protein. By mass spectrometry Ag43 was demonstrated to be glycosylated by addition of heptose residues at several positions in the passenger domain. Glycosylation of Ag43 by the action of the Aah and TibC glycosyltransferases was observed in laboratory strains. Importantly, Ag43 was also found to be glycosylated in a wild-type strain, suggesting that Ag43-glycosylation may be a widespread phenomenon. Glycosylation of Ag43 does not seem to interfere with its self-associating properties. However, the glycosylated form of Ag43 enhances bacterial binding to human cell lines, whereas the nonglycosylated version of Ag43 does not to confer this property.

Shoham, Y., R. Lamed, et al. (1999). "The cellulosome concept as an efficient microbial

strategy for the degradation of insoluble polysaccharides." Trends Microbiol **7**(7): 275-81.

The cellulosome is an extracellular supramolecular machine that can efficiently degrade crystalline cellulosic substrates and associated plant cell wall polysaccharides. The cellulosome arrangement can also promote adhesion to the insoluble substrate, thus providing individual microbial cells with a direct competitive advantage in the utilization of the soluble hydrolysis products.

Sleytr, U. B. and T. J. Beveridge (1999). "Bacterial S-layers." Trends Microbiol **7**(6): 253-60.

S-layers are produced by the self assembly of proteinaceous subunits on the surfaces of prokaryotes, so that planar, monomolecular-thick crystalline lattices are formed. Some archaeal and eubacterial S-layer proteins are glycosylated. These lattices typically have center-to-center spacings of less than 25 nm, which makes them attractive for biomimetic or nanotechnological applications.

Sleytr, U. B., M. Sara, et al. (1986). "Structural and chemical characterization of S-layers of selected strains of Bacillus stearothermophilus and Desulfotomaculum nigrificans." Arch Microbiol **146**(1): 19-24.

The structures, amino acid- and neutral sugar compositions of the crystalline surface layers (S-layers) of four selected strains each of Bacillus stearothermophilus and Desulfotomaculum nigrificans were compared. Among the four strains of each species a remarkable diversity in the molecular weights of the S-layer subunits and in the geometry and constants of the S-layer lattices was apparent. The crystalline arrays included hexagonal (p6), square (p4) and oblique (p2) lattices. In vitro self-assembly of isolated S-layer subunits (or S-layer fragments) led to the formation of flat sheets or open-ended cylindrical assembly products. The amino acid composition of the S-layers exhibited great similarities and was predominantly acidic. With the exception of the S-layers of two strains of B. stearothermophilus (where only traces of neutral sugars could be detected), all other S-layer proteins seemed to be glycosylated. Among these strains significant differences in the amount and composition of the glycan portions were found. Based on this diversity interesting questions may be asked about the biological significance of the carbohydrate units of glycoproteins in prokaryotic organisms.

Southam, G., M. L. Kalmokoff, et al. (1990). "Isolation, characterization, and cellular insertion of the flagella from two strains of the archaebacterium Methanospirillum hungatei." J Bacteriol **172**(6): 3221-8.

In high (45 mM)-phosphate medium, Methanospirillum hungatei strains GP1 and JF1 grew as very long, nonmotile chains of cells that did not possess flagella. However, growth in lower (3 or 30 mM)-phosphate medium resulted in the production of mostly single cells and short chains that were motile by means of two polar tufts of flagella, which transected the multilayered terminal plug of the cell. Electron microscopy of negatively stained whole mounts revealed a flagellar filament diameter of approximately 10 nm. Flagellar filaments were isolated from

either culture fluid or concentrated cell suspensions that were subjected to shearing. Flagellar filaments were sensitive to treatment with both Triton X-100 and Triton X-114 at concentrations as low as 0.1% (vol/vol). The filaments of both strains were composed of two flagellins of Mr 24,000 and 25,000. However, variations in trace element composition of the medium resulted in the production of a third flagellin in strain JF1. This additional flagellin appeared as a ladderlike smear on sodium dodecyl sulfate-polyacylamide gels with a center of intensity of Mr 35,000 and cross-reacted with antisera produced from filaments containing only the Mr-24,000 and -25,000 flagellins. On sodium dodecyl sulfate-polyacrylamide gels, all flagellins stained by the thymol-sulfuric acid and Alcian blue methods, suggesting that they were glycosylated. This was further supported by chemical deglycosylation of the strain JF1 flagellins, which resulted in a reduction in their apparent molecular weight on sodium dodecyl sulfate-polyacylamide gels. Heterologous reactions to sera raised against the flagella from each strain were limited to the Mr-24,000 flagellins.

Stephenson, A. E., H. Wu, et al. (2002). "The Fap1 fimbrial adhesin is a glycoprotein: antibodies specific for the glycan moiety block the adhesion of Streptococcus parasanguis in an in vitro tooth model." Mol Microbiol **43**(1): 147-57.

Streptococcus parasanguis is a primary colonizer of the tooth surface and plays a pivotal role in the formation of dental plaque. The fimbriae of S. parasanguis are important in mediating adhesion to saliva-coated hydroxylapatite (SHA), an in vitro tooth adhesion model. The Fap1 adhesin has been identified as the major fimbrial subunit, and recent studies suggest that Fap1 is a glycoprotein. Monosaccharide analysis of Fap1 purified from the culture supernatant of S. parasanguis indicated the presence of rhamnose, glucose, galactose, N-acetylglucosamine and N-acetylgalactosamine. A glycopeptide moiety was isolated from a pronase digest of Fap1 and purified by immunoaffinity chromatography. The monosaccharide composition of the purified glycopeptide was similar to that of the intact molecule. The functionality of the glycan moiety was determined using monoclonal antibodies (MAbs) specific for the intact Fap1 glycoprotein. These antibodies were grouped into two categories based on their ability to block adhesion of S. parasanguis to SHA and their corresponding specificity for either protein or glycan epitopes of the Fap1 protein. 'Non-blocking' MAb epitopes were mapped to unique protein sequences in the N-terminus of the Fap1 protein using non-glycosylated recombinant Fap1 proteins (rFap1 and drFap1) expressed in Escherichia coli. In contrast, the 'blocking' antibodies did not bind to the recombinant Fap1 proteins, and were effectively competed by the binding to the purified glycopeptide. These data suggest that the 'blocking' antibodies are specific for the glycan moiety and that the adhesion of S. parasanguis is mediated by sugar residues associated with Fap1.

Stoll, D., H. Stalbrand, et al. (1999). "Mannan-degrading enzymes from Cellulomonas fimi." Appl Environ Microbiol **65**(6): 2598-605.

The genes man26a and man2A from Cellulomonas fimi encode mannanase 26A (Man26A) and beta-mannosidase 2A (Man2A), respectively. Mature Man26A is a

secreted, modular protein of 951 amino acids, comprising a catalytic module in family 26 of glycosyl hydrolases, an S-layer homology module, and two modules of unknown function. Exposure of Man26A produced by Escherichia coli to C. fimi protease generates active fragments of the enzyme that correspond to polypeptides with mannanase activity produced by C. fimi during growth on mannans, indicating that it may be the only mannanase produced by the organism. A significant fraction of the Man26A produced by C. fimi remains cell associated. Man2A is an intracellular enzyme comprising a catalytic module in a subfamily of family 2 of the glycosyl hydrolases that at present contains only mammalian beta-mannosidases.

Strobel, G. A., K. W. Talmadge, et al. (1971). "Observations on the structure of the phytotoxic glycopeptide of Corynebacterium sepedonicum." Biochim Biophys Acta **261**(2): 365-74.

Swanson, A. F. and C. C. Kuo (1990). "Identification of lectin-binding proteins in Chlamydia species." Infect Immun **58**(2): 502-7.

Lectin-binding proteins of chlamydiae were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. All three Chlamydia species tested expressed two proteins when whole-elementary-body lysates were reacted with the biotinylated lectin Dolichos biflorus agglutinin. The protein with a molecular mass of 18 kilodaltons (kDa) responded strongly compared with a higher-molecular-mass protein that varied from 27 to 32 kDa with each chlamydia strain tested. Among six lectins tested, including concanavalin A, D. biflorus agglutinin, Ulex europaeus agglutinin, soybean agglutinin, peanut agglutinin, and wheat germ agglutinin, the latter was the only lectin that did not recognize any chlamydial protein. For each lectin that reacted against the elementary body of serovar L2 of Chlamydia trachomatis, the same two peptides, an 18-kDa peptide and a 32-kDa peptide, were revealed. These two polypeptides adhered to HeLa cell surface components. Binding of a lectin to the L2 reticulate body resulted in a reduced response at the 18-kDa peptide. The 18- and 32-kDa peptides were purified from L2 serovar elementary bodies by affinity chromatography. The two proteins isolated from a concanavalin A-agarose column maintained their lectin-binding capacities and elicited hemagglutinating properties against mouse erythrocytes. Periodate oxidation abolished the abilities of the peptides to adhere to any of the lectins tested. These results suggest that these lectin-binding proteins are glycoproteins that may be an essential factor for attachment of chlamydial organisms to host cells.

Swanson, A. F. and C. C. Kuo (1991). "The characterization of lectin-binding proteins of Chlamydia trachomatis as glycoproteins." Microb Pathog **10**(6): 465-73.

The 18 kDa and 32 kDa lectin binding proteins of Chlamydia trachomatis were characterized as glycoproteins by treatments with glycosidases. The proteins of the serovar L2 whole cell lysate were separated by SDS-PAGE and transferred to nitrocellulose paper. After treatment with an enzyme, the proteins were reacted with a biotinylated lectin. Each of the endoglycosidases tested affected

the binding of the lectin to the protein. PNGase F inhibited the binding of Dolichos biflorus agglutinin (DBA), soybean agglutinin (SBA), and Ulex europaeus agglutinin I (UEAI) to both the 18 kDa and 32 kDa proteins. Endoglycosidase F and H inhibited the binding of these lectins to the 32 kDa protein completely and to the 18 kDa protein partially. In the exoglycosidase treatments, alpha-L-fucosidase prevented binding of only UEAI to the two proteins while beta-galactosidase inhibited the binding of SBA. Mannosidase abolished the binding of all the lectins tested. Neuraminidase had no effect. The proteins isolated by electroelution from the excised gels after SDS-PAGE were digested with an endoglycosidase. PNGase F-treated proteins showed a lower molecular weight mobility in which the lectin binding ability was destroyed. Endo-alpha-N-acetylgalactosaminidase had no effect. The polysaccharide stain of isolated proteins with p-phenylenediamine showed a positive reaction. Radiolabeling with [3H]glucosamine did not reveal the 18 kDa and 32 kDa proteins in autoradiography but [3H]galactose did.

Swanson, A. F. and C. C. Kuo (1991). "Evidence that the major outer membrane protein of Chlamydia trachomatis is glycosylated." Infect Immun **59**(6): 2120-5.

The major outer membrane protein (MOMP) of Chlamydia trachomatis was determined to be a glycoprotein on the basis of susceptibility to glycosidase digestion and the presence of carbohydrate by staining and radiolabeling. The MOMP of the serovar L2 organisms was isolated by electroelution from the protein band excised from the gel after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The incubation of MOMP with N-glycosidase F, an endoglycosidase that cleaves the N-glycan, and periodate resulted in two new molecular weight species. While MOMP treated with N-glycosidase F showed a lower-molecular-weight mobility, the periodate-treated MOMP increased in molecular weight. Both treatments abolished the ability of the MOMP to bind to HeLa cell components. In the immunoblot, the reactivity to the monoclonal antibody specific against the C. trachomatis species was preserved. The endoglycosidase specific to O-linked glycan, endo-alpha-N-acetylgalactosaminidase, had no visible effect on the isolated MOMP. Carbohydrate was detected in the MOMP by p-phenylenediamine staining of the protein band in the gel following SDS-PAGE. Autoradiograms of proteins of chlamydial organisms metabolically labeled with [3H]galactose or [3H]glucosamine and separated by SDS-PAGE revealed the MOMP band. The isolated MOMP was shown to bind specifically to concanavalin A, wheat germ agglutinin, and Dolichos biflorus agglutinin in the lectin binding assay. No binding was observed with Ulex europaeus agglutinin I, soybean agglutinin, or Ricinus communis agglutinin.

Swanson, A. F. and C. C. Kuo (1994). "Binding of the glycan of the major outer membrane protein of Chlamydia trachomatis to HeLa cells." Infect Immun **62**(1): 24-8.

Recent studies have shown that the major outer membrane protein (MOMP) of Chlamydia trachomatis is glycosylated. The glycan of the MOMP of C. trachomatis serovar L2 was separated from the glycoprotein with N-glycanase,

reduced with tritiated NaBH4, and tested for its ability to interact with HeLa cells. The [3H]glycan was shown to attach readily to HeLa cells at 25 or 37 degrees C. This process was slower at 4 degrees C. Competition for possibly similar receptor sites on HeLa cells between the glycan and a sugar, an aminosaccharide, or elementary bodies (EBs) was then studied. D-Galactose, D-mannose, or N-acetylglucosamine was shown to reduce the attachment of the glycan to HeLa cells at concentrations of 0.1 to 0.5 M. Sedoheptulose, D-fructose, or sialic acid did not inhibit the binding of glycan to HeLa cells. The presence of at least 100 native or UV-inactivated EBs per HeLa cell interfered with the glycan's ability to bind to HeLa cells. Heat-inactivated EBs did not compete with the glycan for binding. In the reverse situation, nonradiolabeled glycan prevented the EBs from infecting and forming inclusions in HeLa cells. Incubation of [3H]glycan with rabbit immune serum prepared against antigens of whole EB and the MOMP inhibited attachment. In contrast, incubation of glycan with mouse monoclonal antibodies against the protein portion of the MOMP or the chlamydial lipopolysaccharide did not inhibit attachment. These results suggest that the glycan portion of the MOMP is involved in the attachment process of C. trachomatis organisms to HeLa cells.

Swanson, A. F. and C. C. Kuo (1996). "The 18-kDa lectin-binding protein of Chlamydia trachomatis is different from the 18-kDa histone-like protein." FEMS Microbiol Lett **137**(2-3): 189-92.

Five proteins of Chlamydia trachomatis at the 18,000 (18-kDa) molecular mass region were resolved by two-dimensional electrophoresis. Three proteins at 18.2 kDa, pI 6.9, 18.0 kDa, pI 6.3, and 17.9 kDa, pI 6.4 were shown to bind lectin. A fourth protein of 18.0 kDa at pI 10 was the histone-like protein. The fifth protein at 17.9 kDa, pI 7.0 was not characterized.

Sylvestre, P., E. Couture-Tosi, et al. (2002). "A collagen-like surface glycoprotein is a structural component of the Bacillus anthracis exosporium." Mol Microbiol **45**(1): 169-78.

Bacillus anthracis, the aetiological agent of anthrax, is a Gram-positive spore-forming bacterium. The exosporium is the outermost integument surrounding the mature spore. Here, we describe the purification and the characterization of an immunodominant protein of the spore surface. This protein was abundant, glycosylated and part of the exosporium. The amino-terminal sequence was determined and the corresponding gene was identified. It encodes a protein of 382 amino acid residues, the central part of which contains a region of GXX motifs presenting similarity to mammalian collagen proteins. Thus, this collagen-like surface protein was named BclA (for Bacillus collagen-like protein of anthracis). BclA was absent from vegetative cells; it was detected only in spores and sporulating cells. A potential promoter, dependent on the sigma factor sigma(K), which is required for a variety of events late in sporulation, was found upstream from the bclA gene. A bclA deletion mutant was constructed and analysed. Electron microscopy studies showed that BclA is a structural component of the filaments covering the outer layer of the exosporium.

Taguchi, F., R. Shimizu, et al. (2003). "Post-translational modification of flagellin determines the specificity of HR induction." Plant Cell Physiol **44**(3): 342-9.
Flagellin, a constituent of the flagellar filament, is a potent elicitor of hypersensitive cell death in plant cells. Flagellins of Pseudomonas syringae pvs. glycinea and tomato induce hypersensitive cell death in their non-host tobacco plants, whereas those of P. syringae pv. tabaci do not remarkably induce it in its host tobacco plants. However, the deduced amino acid sequences of flagellins from pvs. tabaci and glycinea are identical, indicating that post-translational modification of flagellins plays an important role in determining hypersensitive reaction (HR)-inducibility. To investigate genetically the role of modification of flagellin in HR-induction, biological and phytopathological phenotypes of a flagella-defective Delta fliC mutant and Delta fliC mutants complemented by the introduction of the flagellin gene (fliC) from different pathovars of P. syringae were investigated. The Delta fliC mutant of pv. tabaci lost flagella, motility, the ability to induce HR cell death in non-host tomato cells and virulence toward host tobacco plants, whereas all pv. tabaci complemented by the introduction of the fliC gene of pvs. tabaci, glycinea or tomato recovered all the abilities that the Delta fliC mutant had lost. These results indicate that post-translational modification of flagellins is strongly correlated with the ability to cause HR cell death.

Takamatsu, D., B. A. Bensing, et al. (2004). "Four proteins encoded in the gspB-secY2A2 operon of Streptococcus gordonii mediate the intracellular glycosylation of the platelet-binding protein GspB." J Bacteriol **186**(21): 7100-11.
Platelet binding by Streptococcus gordonii strain M99 is mediated predominantly by the cell surface glycoprotein GspB. This adhesin consists of a putative N-terminal signal peptide, two serine-rich regions (SRR1 and SRR2), a basic region between SRR1 and SRR2, and a C-terminal cell wall anchoring domain. The glycosylation of GspB is mediated at least in part by Gly and Nss, which are encoded in the secY2A2 locus immediately downstream of gspB. This region also encodes two proteins (Gtf and Orf4) that are required for the expression of GspB but whose functions have not been delineated. In this study, we further characterized the roles of Gly, Nss, Gtf, and Orf4 by investigating the expression and glycosylation of a series of glutathione S-transferase-GspB fusion proteins in M99 and in gly, nss, gtf, and orf4 mutants. Compared with fusion proteins expressed in the wild-type background, fusion proteins expressed in the mutant strain backgrounds showed altered electrophoretic mobility. In addition, the fusion proteins formed insoluble aggregates in protoplasts of the gtf and orf4 mutants. Glycan detection and lectin blot analysis revealed that SRR1 and SRR2 were glycosylated but that the basic region was unmodified. When the fusion protein was expressed in Escherichia coli, glycosylation of this protein was observed only in the presence of both gtf and orf4. These results demonstrate that Gly, Nss, Gtf, and Orf4 are all involved in the intracellular glycosylation of SRRs. Moreover, Gtf and Orf4 are essential for glycosylation, which in turn is important for the solubility of GspB.

Taku, A. and D. P. Fan (1976). "Purification and properties of a protein factor stimulating peptidoglycan synthesis in toluene- and Licl-treated Bacillus megaterium cells." J Biol Chem **251**(7): 1889-95.

A protein factor, called PG-I, can be solubilized from toluene-treated Bacillus megaterium cells by LiCl extraction. After LiCl extraction, peptidoglycan synthesis by the toluene-treated cells is decreased. Protein PG-I can be added back to the extracted cells to stimulate peptidoglycan synthesis. This factor has now been purified 124-fold. It has a molecular weight of 42,000 as estimated by Sephadex gel filtration in the presence of 0.4 M KCl and 52,000 as determined by sodium dodecyl sulfate disc gel electrophoresis. Periodate-Schiff staining of the polyacrylamide gel indicates that factor PG-I is a glycoprotein. The reconstitution of LiCl-extracted cells requires Mg^{2+} with an apparent Km of 1.9 X 10(-3) M. The Mg^{2+} ions can be replaced by Ca^{2+} and by Mn^{2+} ions to some extent; Zn^{2+} and Cu^{2+} ions had no effect. The available data suggest that factor PG-I is essential for peptidoglycan synthesis and requires at least one thiol group for stimulatory activity.

Tamaru, Y., T. Araki, et al. (1995). "Purification and characterization of an extracellular beta-1,4-mannanase from a marine bacterium, Vibrio sp. strain MA-138." Appl Environ Microbiol **61**(12): 4454-8.

A beta-mannanase (EC 3.2.1.78) from Vibrio sp. strain MA-138 was purified by ammonium sulfate precipitation and several chromatographic procedures including gel filtration, adsorption, and ion-exchange chromatographies. The final ion-exchange chromatography Mono Q yielded one major active fraction and three minor active fractions. The major active fraction was purified to homogeneity on the basis of native polyacrylamide gel electrophoresis (PAGE). This purified enzyme was identified as a glycoprotein by periodic acid-Schiff staining and a monomeric protein with a molecular mass of 49 kDa by sodium dodecyl sulfate-PAGE. The pI of the enzyme was 3.8. The purified enzyme exhibited maximal activity at pH 6.5 and 40 degrees C and hydrolyzed at random the internal beta-1,4-mannosidic linkages in beta-mannan to give various sizes of oligosaccharides. The first 20 N-terminal amino acid sequence of the purified enzyme showed high homology with the N-terminal region of beta-mannanase from Streptomyces lividans 66.

Tamborrini, M., M. Holzer, et al. "Anthrax spore detection by a luminex assay based on monoclonal antibodies that recognize anthrose-containing oligosaccharides." Clin Vaccine Immunol **17**(9): 1446-51.

The similarity of endospore surface antigens between bacteria of the Bacillus cereus group complicates the development of selective antibody-based anthrax detection systems. The surface of B. anthracis endospores exposes a tetrasaccharide containing the monosaccharide anthrose. Anti-tetrasaccharide monoclonal antibodies (MAbs) and anti-anthrose-rhamnose disaccharide MAbs were produced and tested for their fine specificities in a direct spore enzyme-linked immunosorbent assay (ELISA) with inactivated spores of a broad spectrum of B. anthracis strains and related species of the Bacillus genus.

Although the two sets of MAbs had different fine specificities, all of them recognized the tested B. anthracis strains and showed only a limited cross-reactivity with two B. cereus strains. The MAbs were further tested for their ability to be implemented in a highly sensitive and specific bead-based Luminex assay. This assay detected spores from different B. anthracis strains and two cross-reactive B. cereus strains, correlating with the results obtained in direct spore ELISA. The Luminex assay (detection limit 10(3) to 10(4) spores per ml) was much more sensitive than the corresponding sandwich ELISA. Although not strictly specific for B. anthracis spores, the developed Luminex assay represents a useful first-line screening tool for the detection of B. anthracis spores.

Tang, G. and K. P. Mintz "Glycosylation of the collagen adhesin EmaA of Aggregatibacter actinomycetemcomitans is dependent upon the lipopolysaccharide biosynthetic pathway." J Bacteriol **192**(5): 1395-404.

The human oropharyngeal pathogen Aggregatibacter actinomycetemcomitans synthesizes multiple adhesins, including the nonfimbrial extracellular matrix protein adhesin A (EmaA). EmaA monomers trimerize to form antennae-like structures on the surface of the bacterium, which are required for collagen binding. Two forms of the protein have been identified, which are suggested to be linked with the type of O-polysaccharide (O-PS) of the lipopolysaccharide (LPS) synthesized (G. Tang et al., Microbiology 153:2447-2457, 2007). This association was investigated by generating individual mutants for a rhamnose sugar biosynthetic enzyme (rmlC; TDP-4-keto-6-deoxy-d-glucose 3,5-epimerase), the ATP binding cassette (ABC) sugar transport protein (wzt), and the O-antigen ligase (waaL). All three mutants produced reduced amounts of O-PS, and the EmaA monomers in these mutants displayed a change in their electrophoretic mobility and aggregation state, as observed in sodium dodecyl sulfate (SDS)-polyacrylamide gels. The modification of EmaA with O-PS sugars was suggested by lectin blots, using the fucose-specific Lens culinaris agglutinin (LCA). Fucose is one of the glycan components of serotype b O-PS. The rmlC mutant strain expressing the modified EmaA protein demonstrated reduced collagen adhesion using an in vitro rabbit heart valve model, suggesting a role for the glycoconjugant in collagen binding. These data provide experimental evidence for the glycosylation of an oligomeric, coiled-coil adhesin and for the dependence of the posttranslational modification of EmaA on the LPS biosynthetic machinery in A. actinomycetemcomitans.

Tani, Y., M. Tani, et al. (1997). "Extracellular 37-kDa antigenic protein from Actinobacillus actinomycetemcomitans induces TNF-alpha, IL-1 beta, and IL-6 in murine macrophages." J Dent Res **76**(9): 1538-47.

The extracellular antigens of Actinobacillus actinomycetemcomitans Y4 (serotype b) contain a 37-kDa protein which is a major target for IgGs from patients suffering from severe alveolar bone loss. Since the 37-kDa protein has not been studied sufficiently, our investigation focused on its characteristics, e.g., its localization, specificity, and whether it directly stimulates macrophages to produce cytokines. The 37-kDa protein was purified from the culture supernatant

of the Y4 strain by means of chromatofocusing and gel filtration. The 37-kDa protein is a unique glycoprotein which forms immune complexes with monoclonal antibodies against rhamnose-fucose polysaccharide. Patients with A. actinomycetemcomitans-associated periodontitis had higher antibody titers to the purified 37-kDa protein than healthy subjects ($p < 0.001$). Anti-37-kDa protein antibodies recognized a 37-kDa band in the cytosolic, ribosomal, and total membrane fractions from Y4 cells. Extracellular substances from other strains of A. actinomycetemcomitans (serotypes a and c) also reacted in the Western blots, but Haemophilus spp. or several periodontopathic bacteria did not. These results suggested that the 37-kDa protein is a cytosolic protein that is passed through the cell membrane, and its protein portion is specific for A. actinomycetemcomitans but common to serotypes. This protein induced Il-1 beta, Il-6, and TNF-alpha release from murine macrophages. The Il-6-inducing activity of the 37-kDa protein was higher than that of LPS. These findings suggested that the 37-kDa protein which is released from live cells plays a role in A. actinomycetemcomitans-associated periodontitis, as antigen inducing the release of inflammatory cytokines which are associated with alveolar bone loss.

Tian, X. X., A. Li, et al. (2000). "Isolation and identification of poly-alpha-(1-->4)-linked 3-O-methyl-D-mannopyranose from a hot-water extract of Mycobacterium vaccae." Carbohydr Res **324**(1): 38-44.

A polysaccharide around 3.6 kDa has been identified as the major carbohydrate moiety of a antineoplastic protein-polysaccharide complex (PS4A) obtained by boiling intact cells of Mycobacterium vaccae in water. 1H and 13C NMR spectra of this polysaccharide suggested it was a highly homogeneous polymer composed substantially of one monomer, probably an alpha-linked O-methylated mannose. Comparison of the COSY spectra of the original and acetylated polymer indicated that the glycosidic linkage and the methyl ether were interchangeable, at O-3 and O-4. Further study demonstrated that the benzyolated hydrolysate of the polymer was 1,2,4,6-tetra-O-benzoyl-3-O-methyl-beta-mannopyranose. The hydrolysate was 3-O-methyl-alpha, beta-mannopyranose and the polymer was therefore poly-alpha-(1-->4)-linked 3-O-methyl-D-mannopyranose. This conclusion was further confirmed with an authentic sample of the monomer, which had spectral data identical to those of the hydrolyzate and co-eluted from an ion-exchange HPLC with the major sugar in the hydrolysate.

Tian, X. X., A. Li, et al. (1999). "Isolation and biological activities of an antineoplastic protein-polysaccharide complex (PS4A) obtained from Mycobacterium vaccae." Anticancer Res **19**(1A): 237-43.

A mixture of water-soluble protein-polysaccharides (PS4A) was isolated by boiling intact cells of Mycobacterium vaccae, a fast growing mycobacterium. Sephadex G-75 column chromatography of the crude extract separated the biologically active high molecular weight (> 50 kDa) fraction (in the void volume) from the low molecular weight degradation products. Compositional analysis demonstrated that PS4A contained protein and polysaccharide in a ratio of

approximately 1.5 to 1, but no lipids were detected. The antineoplastic activity was tested in vivo by a S-180 murine sarcoma model using female CFW mice. The immunostimulating activity was tested in vitro using murine peritoneal macrophages isolated from BALB/C mice. The results demonstrated that PS4A significantly decreased tumor incidence in vivo and produced activation of murine peritoneal macrophages. However, the antineoplastic activity was only attributable to the high molecular weight fraction of the protein-polysaccharide complex. The low molecular weight fraction had no antineoplastic activity in vivo despite stimulation of TNF-alpha production in vitro. In vitro experiments also demonstrated that although all PS4A components significantly increased TNF-alpha production by macrophages, the high molecular weight fraction stimulated more IL-1 production, indicating a better immunostimulating activity.

Todd, S. J., A. J. Moir, et al. (2003). "Genes of Bacillus cereus and Bacillus anthracis encoding proteins of the exosporium." J Bacteriol **185**(11): 3373-8.

The exosporium is the outermost layer of spores of Bacillus cereus and its close relatives Bacillus anthracis and Bacillus thuringiensis. For these pathogens, it represents the surface layer that makes initial contact with the host. To date, only the BclA glycoprotein has been described as a component of the exosporium; this paper defines 10 more tightly associated proteins from the exosporium of B. cereus ATCC 10876, identified by N-terminal sequencing of proteins from purified, washed exosporium. Likely coding sequences were identified from the incomplete genome sequence of B. anthracis or B. cereus ATCC 14579, and the precise corresponding sequence from B. cereus ATCC 10876 was defined by PCR and sequencing. Eight genes encode likely structural components (exsB, exsC, exsD, exsE, exsF, exsG, exsJ, and cotE). Several proteins of the exosporium are related to morphogenetic and outer spore coat proteins of B. subtilis, but most do not have homologues in B. subtilis. ExsE is processed from a larger precursor, and the CotE homologue appears to have been C-terminally truncated. ExsJ contains a domain of GXX collagen-like repeats, like the BclA exosporium protein of B. anthracis. Although most of the exosporium genes are scattered on the genome, bclA and exsF are clustered in a region flanking the rhamnose biosynthesis operon; rhamnose is part of the sugar moiety of spore glycoproteins. Two enzymes, alanine racemase and nucleoside hydrolase, are tightly adsorbed to the exosporium layer; they could metabolize small molecule germinants and may reduce the sensitivity of spores to these, limiting premature germination.

Tomoeda, M., M. Inuzuka, et al. (1975). "Bacterial sex pili." Prog Biophys Mol Biol **30**(1): 23-56.

Turner, M. A., F. Arellano, et al. (1991). "Components of ice nucleation structures of bacteria." J Bacteriol **173**(20): 6515-27.

Nonprotein components attached to the known protein product of the inaZ gene of Pseudomonas syringae have been identified and shown to be necessary for the most efficient ice nucleation of supercooled H2O. Previous studies have

shown that cultures of Ina+ bacteria have cells with three major classes of ice-nucleating structures with readily differentiated activities. Further, some cells in the culture have nucleating activities intermediate between those of the different classes and presumably have structures that are biosynthetic intermediates between those of the different classes. Since these structures cannot be readily isolated and analyzed, their components have been identified by the use of specific enzymes or chemical probes, by direct incorporation of labeled precursors, and by stimulation of the formation of specific classes of freezing structures by selective additions to the growth medium. From these preliminary studies it appears that the most active ice nucleation structure (class A) contains the ice nucleation protein linked to phosphatidylinositol and mannose, probably as a complex mannan, and possibly glucosamine. These nonprotein components are characteristic of those used to anchor external proteins to cell membranes of eucaryotic cells and suggest that a similar but not identical anchoring mechanism is required for efficient ice nucleation structure. The class B structure has been found to contain protein presumably linked to the mannan and glucosamine moieties but definitely not to the phosphatidylinositol. The class C structure, which has the poorest ice nucleation activity, appears to be the ice nucleation protein linked to a few mannose residues and to be partially imbedded in the outer cell membrane.

Upreti, R. K., M. Kumar, et al. (2003). "Bacterial glycoproteins: functions, biosynthesis and applications." Proteomics **3**(4): 363-79.

Although widely distributed in eukaryotic cells glycoproteins appear to be rare in prokaryotic organisms. The prevalence of the misconception that bacteria do not glycosylate their proteins has been a subject matter of discussion for a long time. Glycoconjugates that are linked to proteins or peptides, generated by the ribosomal translational mechanism have been reported only in the last two to three decades in a few prokaryotic organisms. Most studied prokaryotic glycoproteins are the S-layer glycoproteins of Archeabacteria. Apart from these, membrane-associated, surface-associated, secreted glycoproteins and exoenzymes glycoproteins are also well documented in both, Archea and Eubacteria. From the recent literature, it is now clear that prokaryotes are capable of glycosylating proteins. In general, prokaryotes are deprived of the cellular organelles required for glycosylation. In prokaryotes many different glycoprotein structures have been observed that display much more variation than that observed in eukaryotes. Besides following similar mechanisms in the process of glycosylation, prokaryotes have also been shown to use mechanisms that are different from those found in eukaryotes. The knowledge pertaining to the functional aspects of prokaryotic glycoproteins is rather scarce. This review summarizes developments and understanding relating to characteristics, synthesis, and functions of prokaryotic glycoproteins. An extensive summary of glycosylation that has been reported to occur in bacteria has also been tabulated. Various possible applications of these diverse biomolecules in biotechnology, vaccine development, pharmaceutics and diagnostics are also touched upon.

Uthandi, S., B. Saad, et al. "LccA, an archaeal laccase secreted as a highly stable glycoprotein into the extracellular medium by Haloferax volcanii." Appl Environ Microbiol **76**(3): 733-43.

Laccases couple the oxidation of phenolic compounds to the reduction of molecular oxygen and thus span a wide variety of applications. While laccases of eukaryotes and bacteria are well characterized, these enzymes have not been described in archaea. Here, we report the purification and characterization of a laccase (LccA) from the halophilic archaeon Haloferax volcanii. LccA was secreted at high levels into the culture supernatant of a recombinant H. volcanii strain, with peak activity (170 +/- 10 mU.ml(-)(1)) at stationary phase (72 to 80 h). LccA was purified 13-fold to an overall yield of 72% and a specific activity of 29.4 U.mg(-)(1) with an absorbance spectrum typical of blue multicopper oxidases. The mature LccA was processed to expose an N-terminal Ala after the removal of 31 amino acid residues and was glycosylated to 6.9% carbohydrate content. Purified LccA oxidized a variety of organic substrates, including bilirubin, syringaldazine (SGZ), 2,2,-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and dimethoxyphenol (DMP), with DMP oxidation requiring the addition of CuSO(4). Optimal oxidation of ABTS and SGZ was at 45 degrees C and pH 6 and pH 8.4, respectively. The apparent K(m) values for SGZ, bilirubin, and ABTS were 35, 236, and 670 muM, with corresponding k(cat) values of 22, 29, and 10 s(-)(1), respectively. The purified LccA was tolerant of high salt, mixed organosolvents, and high temperatures, with a half-life of inactivation at 50 degrees C of 31.5 h.

Van Rijssel, M., G. J. Gerwig, et al. (1993). "Isolation and characterization of an extracellular glycosylated protein complex from Clostridium thermosaccharolyticum with pectin methylesterase and polygalacturonate hydrolase activity." Appl Environ Microbiol **59**(3): 828-36.

An extracellular protein complex was isolated from the supernatant of a pectin-limited continuous culture of Clostridium thermosaccharolyticum Haren. The complex possessed both pectin methylesterase (EC 3.1.1.11) and exo-poly-alpha-galacturonate hydrolase (EC 3.2.1.82) activity and produced digalacturonate from the nonreducing end of the pectin chain. The protein consisted of 230- and 25-kDa subunits. The large subunit contained 10% (wt/wt) sugars (N-acetylgalactosamine and galactose). Under physiological conditions both activities acted in a coordinated manner: the ratio between methanol and digalacturonate released during degradation was constant and equal to the degree of esterification of the pectin used. Prolonged incubation of the enzyme with pectin led to a nondialyzable fraction that was enriched in neutral sugars, such as arabinose, rhamnose, and galactose; the high rhamnose/galacturonic acid ratio was indicative of hairy region-like structures. The smallest substrate utilized by the hydrolase was a tetragalacturonate. Vmax with oligogalacturonates increased with increasing chain length. The Km and Vmax for the polygalacturonate hydrolase with citrus pectate as a substrate were 0.8 g liter-1 and 180 mumol min-1 mg of protein-1, respectively. The Km and Vmax for the esterase with citrus pectin as a substrate were 1.2 g liter-1 and 440 mumol

min-1 mg of protein-1, respectively. The temperature optima for the hydrolase and esterase were 70 and 60 degrees C, respectively. Both enzyme activities were stable for more than 1 h at 70 degrees C. The exo-polygalacturonate hydrolase of Clostridium thermosulfurogenes was partially purified while the methylesterase was also copurified.

Vik, A., F. E. Aas, et al. (2009). "Broad spectrum O-linked protein glycosylation in the human pathogen Neisseria gonorrhoeae." Proc Natl Acad Sci U S A **106**(11): 4447-52.

Protein glycosylation is an important element of biologic systems because of its significant effects on protein properties and functions. Although prominent within all domains of life, O-linked glycosylation systems modifying serine and threonine residues within bacteria and eukaryotes differ substantially in target protein selectivity. In particular, well-characterized bacterial systems have been invariably dedicated to modification of individual proteins or related subsets thereof. Here we characterize a general O-linked glycosylation system that targets structurally and functionally diverse groups of membrane-associated proteins in the gram-negative bacterium Neisseria gonorrhoeae, the etiologic agent of the human disease gonorrhea. The 11 glycoproteins identified here are implicated in activities as varied as protein folding, disulfide bond formation, and solute uptake, as well as both aerobic and anaerobic respiration. Along with their common trafficking within the periplasmic compartment, the protein substrates share quasi-related domains bearing signatures of low complexity that were demonstrated to encompass sites of glycan occupancy. Thus, as in eukaryotes, the broad scope of this system is dictated by the relaxed specificity of the glycan transferase as well as the bulk properties and context of the protein-targeting signal rather than by a strict amino acid consensus sequence. Together, these findings reveal previously unrecognized commonalities linking O-linked protein glycosylation in distantly related life forms.

Wakai, H., S. Nakamura, et al. (1997). "Cloning and sequencing of the gene encoding the cell surface glycoprotein of Haloarcula japonica strain TR-1." Extremophiles **1**(1): 29-35.

The triangular disk-shaped halophilic archaeon Haloarcula japonica strain TR-1 has a glycoprotein on its cell surface. The complete gene encoding the cell surface glycoprotein (CSG) was cloned and sequenced. The gene has an open reading frame of 2586 bp, and a potential archaeal promoter sequence approximately 150 bp upstream of the ATG initiation codon. The mature CSG is composed of 828 amino acids and is preceded by a signal sequence of 34 amino acid residues. A hydropathy analysis showed a hydrophobic stretch at the C-terminus, that probably serves as a transmembrane domain. The amino acid sequence of the Ha. japonica CSG showed 52.1% and 43.2% identities to those from the Halobacterium halobium and Haloferax volcanii CSGs, respectively. Five potential N-glycosylation sites were found in the mature Ha. japonica CSG, sites that were distinctly different from those in Hb. halobium and Hf. volcanii. The Ha. japonica CSG gene was expressed in Escherichia coli.

Waller, L. N., M. J. Stump, et al. (2005). "Identification of a second collagen-like glycoprotein produced by Bacillus anthracis and demonstration of associated spore-specific sugars." J Bacteriol **187**(13): 4592-7.

Certain carbohydrates (rhamnose, 3-O-methyl rhamnose, and galactosamine) have been demonstrated to be present in Bacillus anthracis spores but absent in vegetative cells. Others have demonstrated that these spore-specific sugars are constituents of the glycoprotein BclA. In the current work, spore extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A second collagen-like glycoprotein, BclB, was identified in B. anthracis. The protein moiety of this glycoprotein was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MS) and the carbohydrate components by gas chromatography-mass spectrometry and tandem mass spectrometry. Spore-specific sugars were also demonstrated to be components of BclB.

Webster, J. R., S. J. Reid, et al. (1981). "Purification and Characterization of an Autolysin from Clostridium acetobutylicum." Appl Environ Microbiol **41**(2): 371-4.

A proteinaceous substance with antibiotic-like activity, resembling that of a bacteriocin, was isolated from an industrial-scale acetone-butanol fermentation of Clostridium acetobutylicum. The substance, purified by acetone precipitation, diethylaminoethyl cellulose chromatography, and polyacrylamide gel electrophoresis, was characterized as a glycoprotein with a molecular weight of 28,000. The glycoprotein was partially inactivated by certain protease enzymes. It had no effect on deoxyribonucleic acid, ribonucleic acid, or protein synthesis, and it did not result in the loss of intracellular adenosine triphosphate. The glycoprotein lysed sodium dodecyl sulfate-treated cells and cell wall preparations, and therefore it is referred to as an autolysin. The autolysin gene appeared to be chromosomal since plasmid deoxyribonucleic acid was not detected in the C. acetobutylicum strain.

Wehmeier, S., A. S. Varghese, et al. (2009). "Glycosylation of the phosphate binding protein, PstS, in Streptomyces coelicolor by a pathway that resembles protein O-mannosylation in eukaryotes." Mol Microbiol **71**(2): 421-33.

Previously mutations in a putative protein O-mannosyltransferase (SCO3154, Pmt) and a polyprenol phosphate mannose synthase (SCO1423, Ppm1) were found to cause resistance to phage, phiC31, in the antibiotic producing bacteria Streptomyces coelicolor A3(2). It was proposed that these two enzymes were part of a protein O-glycosylation pathway that was necessary for synthesis of the phage receptor. Here we provide the evidence that Pmt and Ppm1 are indeed both required for protein O-glycosylation. The phosphate binding protein PstS was found to be glycosylated with a trihexose in the S. coelicolor parent strain, J1929, but not in the pmt(-) derivative, DT1025. Ppm1 was necessary for the transfer of mannose to endogenous polyprenol phosphate in membrane preparations of S. coelicolor. A mutation in ppm1 that conferred an E218V substitution in Ppm1 abolished mannose transfer and glycosylation of PstS. Mass spectrometry analysis of extracted lipids showed the presence of a

glycosylated polyprenol phosphate (PP) containing nine repeated isoprenyl units (C(45)-PP). S. coelicolor membranes were also able to catalyse the transfer of mannose to peptides derived from PstS, indicating that these could be targets for Pmt in vivo.

Weinberg, M. V., G. J. Schut, et al. (2005). "Cold shock of a hyperthermophilic archaeon: Pyrococcus furiosus exhibits multiple responses to a suboptimal growth temperature with a key role for membrane-bound glycoproteins." J Bacteriol **187**(1): 336-48.

The hyperthermophilic archaeon, Pyrococcus furiosus, was grown on maltose near its optimal growth temperature, 95 degrees C, and at the lower end of the temperature range for significant growth, 72 degrees C. In addition, cultures were shocked by rapidly dropping the temperature from 95 to 72 degrees C. This resulted in a 5-h lag phase, during which time little growth occurred. Transcriptional analyses using whole-genome DNA microarrays representing 2,065 open reading frames (ORFs) in the P. furiosus genome showed that cells undergo three very different responses at 72 degrees C: an early shock (1 to 2 h), a late shock (5 h), and an adapted response (occurring after many generations at 72 degrees C). Each response involved the up-regulation in the expression of more than 30 ORFs unique to that response. These included proteins involved in translation, solute transport, amino acid biosynthesis, and tungsten and intermediary carbon metabolism, as well as numerous conserved-hypothetical and/or membrane-associated proteins. Two major membrane proteins were evident after one-dimensional sodium dodecyl sulfate-gel analysis of cold-adapted cells, and staining revealed them to be glycoproteins. Their cold-induced expression evident from the DNA microarray analysis was confirmed by quantitative PCR. Termed CipA (PF0190) and CipB (PF1408), both appear to be solute-binding proteins. While the archaea do not contain members of the bacterial cold shock protein (Csp) family, they all contain homologs of CipA and CipB. These proteins are also related phylogenetically to some cold-responsive genes recently identified in certain bacteria. The Cip proteins may represent a general prokaryotic-type cold response mechanism that is present even in hyperthermophilic archaea.

Wimmer, B., F. Lottspeich, et al. (1997). "A novel type of thermostable alpha-D-glucosidase from Thermoanaerobacter thermohydrosulfuricus exhibiting maltodextrinohydrolase activity." Biochem J **328 (Pt 2)**: 581-6.

An alpha-glucosidase with the ability to attack polymeric substrates was purified to homogeneity from culture supernatants of Thermoanaerobacter thermohydrosulfuricus DSM 567. The enzyme is apparently a glycoprotein with a molecular mass of 160 kDa. Maximal activity is observed between pH5 and 7 at 75 degrees C. The alpha-glucosidase is active towards p-nitrophenyl-alpha-D-glucoside, maltose, malto-oligosaccharides, starch and pullulan. Highest activity is displayed towards the disaccharide maltose. In addition to glucose, maltohexaose and maltoheptaose can be detected as the initial products of starch hydrolysis. After short incubations of pullulan, glucose is found as the only

product. At high substrate concentrations, maltose and malto-oligosaccharide, but not glucose, are used as acceptors for glucosyl-transfer. These findings indicate that the T. thermohydrosulfuricus enzyme represents a novel type of alpha-glucosidase exhibiting maltase, glucohydrolase and 'maltodextrinohydrolase' activity.

Wyss, C. (1998). "Flagellins, but not endoflagellar sheath proteins, of Treponema pallidum and of pathogen-related oral spirochetes are glycosylated." Infect Immun **66**(12): 5751-4.

Glycosylation of the flagellar core proteins (FlaBs) was detected in Treponema pallidum Nichols and in the type or reference strains of seven oral Treponema species. In several nonmotile strains of oral treponemes, the FlaBs were undetectable by both antibody and glycan staining. In contrast, a spontaneous low-motility variant of T. vincenti poundi-related strain RitzA, OMZ 305A, lacked the flagellar sheath protein (FlaA) and the two glycan-staining FlaB bands of the wild type, but antibody labeling revealed a novel FlaB band with a lower relative molecular weight. A ca. 38-kDa component of isolated endoflagella of T. vincentii OMZ 800 was identified on Western blots as FlaA by monoclonal antibody (MAb) H9-2, which specifically labels the 37-kDa FlaA protein of T. pallidum. Glycan and H9-2 labeling patterns similar to those of T. pallidum were observed in whole-cell extracts of T. medium G7201 and of 10 strains classified as T. vincentii and as two T. vincentii-related taxons. These four groups were thus identified as cultivable pathogen (T. pallidum)-related oral spirochetes as defined by labeling with MAb H9-2. No H9-2 MAb-reactive component could be detected in T. amylovorum, T. denticola, T. maltophilum, T. pectinovorum, and the three subspecies of T. socranskii.

Yamaguchi, T., K. Kasamo, et al. (1998). "Preparation and characterization of an Actinomyces naeslundii aggregation factor that mediates coaggregation with Porphyromonas gingivalis." J Periodontal Res **33**(8): 460-8.

Intergeneric coaggregation is responsible for the complexity of the microbiota in human dental plaque and is believed to be important in the initial bacterial colonization of the human oral cavity. Actinomyces naeslundii, an early colonizer of the tooth surface, may enhance subsequent colonization by Porphyromonas gingivalis which is associated with adult periodontitis. The purpose of this study was to isolate and characterize the A. naeslundii aggregation factor (AnAF) that mediates coaggregation with P. gingivalis. AnAF was isolated from A. naeslundii sonic extract (SE) by gel filtration on a Sephacryl S-400HR, by hydrophobic interaction chromatography on a HiTrap Octyl Sepharose 4FF, and by ion exchange chromatography on a HiTrap Q. The specific activity increased 12-fold with a yield of 2.5%. SDS-PAGE analysis of AnAF revealed a protein band of high molecular weight in excess of 200 kDa. Carbohydrate was detected as the only material coinciding with the protein band, indicating that the AnAF was a glycoprotein. Immunoblotting analysis indicated that AnAF directly bound to P. gingivalis cells. AnAF was sensitive to sodium metaperiodate treatment but not to heat or protease treatments. These results suggest that the AnAF carbohydrate

component mediated coaggregation with P. gingivalis cells. AnAF also inhibited coaggregation with other periodontal disease-associated bacteria such as Prevotella intermedia, Fusobacterium nucleatum, Capnocytophaga ochracea, but not streptococci.

Yang, L. L. and A. Haug (1979). "Purification and partial characterization of a procaryotic glycoprotein from the plasma membrane of Thermoplasma acidophilum." Biochim Biophys Acta **556**(2): 265-77.

The obligate, thermophilic, acidophilic mycoplasma, Thermoplasma acidophilum, grows optimally at 56 degrees C and pH 2.0. Its plasma membrane possessed 21--22 protein bands that were resolved by polyacrylamide gel electrophoresis. One major membrane protein, molecular weight 152 000, which stained for carbohydrate with periodic acid-Schiff reagent, accounted for 32% (w/w) of the total membrane proteins. It was isolated and further purified by concanavalin A affinity chromatography. The carbohydrate content amounted to less than 10% (w/w) compared to that of the entire glycoprotein. The carbohydrate moiety consisted mainly of mannose residues with branched alpha 1 leads to 2 linkages at the non-reducing ends of the glycopeptide as determined by permethylation followed by gas chromatography-mass spectrometry analysis. The reducing end was an N-glycosidic linkage between asparagine and N-acetylglucosamine. The amino acid composition of this glycoprotein showed 62 mol% hydrophobic residues, while the acidic amino acid content contributed 9 mol% more than that of the basic amino acids. The existence of membrane glycoproteins in the procaryotic, wall-less T. acidophilum may provide a protective coat for the plasma membrane. The stereochemistry and the conformation of the carbohydrate chains, in conjunciton with water turgor, may contribute to the rigidity of the membrane and the cation binding.

Yao, R., A. J. Macario, et al. (1992). "Immunochemical differences among Methanosarcina mazei S-6 morphologic forms." J Bacteriol **174**(14): 4683-8.

Methanosarcinae are the only archaeobacteria known to undergo major morphologic changes during growth involving unicellular and multicellular forms, and Methanosarcina mazei S-6 is the only strain for which three distinct forms, packets, single cells, and lamina, have so far been observed. It is reported that two pairs of these forms, either packets and single cells or single cells and lamina, grew and interconverted in medium with the same composition, Ca2+ and Mg2+ concentrations, and growth substrate, and that the two forms in each pair displayed distinctive differences revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting, the same growth medium-substrate notwithstanding.

Yoshida, J., M. Yoshimura, et al. (1985). "Purification and characterization of an antitumor principle from Streptococcus hemolyticus, Su strain." Jpn J Cancer Res **76**(3): 213-23.

An antitumor principle (SAGP) has been purified from cell-free crude extract (CE) of group A Streptococcus hemolyticus, Su strain. The antitumor activity of each

fraction was evaluated by measuring the in vitro growth inhibitory effect on transformed hamster embryonic lung cells (THEL). CE was subjected to thermal treatment, streptomycin precipitation, ammonium sulfate precipitation, and chromatography on octyl-Sepharose CL-4B, DEAE-cellulose, and Sephadex G-200 in that order. The active fraction from the last chromatography was dialyzed against distilled water. The resulting precipitate was removed and the supernatant was lyophilized (SAGP). SAGP is a homogeneous glycoprotein as judged by polyacrylamide gel electrophoresis, gel filtration, immunodiffusion, and PAS-staining. The molecular weight of SAGP was determined to be 140,000 to 150,000 by the gel filtration technique. SAGP is composed of subunits, each of which has a molecular weight of about 50,000. The isoelectric point of SAGP is 4.3. Amino acid analysis revealed an abundance of aspartic and glutamic acid residues in SAGP. The 50% growth inhibitory dose of SAGP on THEL was 0.062 micrograms/ml. Intraperitoneal administration of SAGP (20 mg/kg/day X 4) to ICR mice bearing Ehrlich ascites carcinoma cells increased their life span to 254% of the control.

Yoshida, S., Y. Sako, et al. (1998). "Cloning, sequence analysis, and expression in Escherichia coli of a gene coding for an enzyme from Bacillus circulans K-1 that degrades guar gum." Biosci Biotechnol Biochem **62**(3): 514-20.

A 2,048-bp nucleotide sequence containing a gene coding for an enzyme that degraded guar gum from Bacillus circulans K-1 was identified by polymerase chain reaction walking. This G-gene consisted of 1,551 nucleotides coding for a protein with Mr 55,242. The enzyme was overexpressed in Escherichia coli JM109 cells by the cloning the G-gene downstream of the lac Z promoter of pUC19. The molecular mass of recombinant G-enzyme estimated by SDS-PAGE was 62 KDa, close to that from strain K-1. Analysis of the recombinant enzyme showed GalNAc, Xyl, GlcNAc, Man, Glc, and Gal to account for 1.7%, 14.4%, 6.1%, 3.2%, 54.2%, and 10.4%, respectively, of the total monosaccharides. Polyacrylamide gel electrophoresis of this enzyme with staining gave a red band. The results suggested that the sugars accounted for the differences in the molecular masses. The recombinant enzyme had two kinds of N-terminal sequences, Thr-Met-Ile-Thr-Pro-Ser-Phe-Ala-Ser-Gly-Phe-Tyr-Val-Ile and Ile-Thr-Pro-Ser-Phe-Ala-Ser-Gly-Phe-Tyr-Val-Ile-Gly-Thr. Comparison of these sequences with the deduced N-terminal sequence coded for the G-gene showed that the amino acid, first Met, of the lac Z gene or the next residues Thr-Met in the recombinant enzyme were absent in the native enzyme. Methionines near and at the N-terminus of the mature protein probably were digested by methionine aminopeptidases of E. coli after translation. The properties of recombinant G-enzyme were similar to those of the enzyme from K-1 cells.

Zaidi, S. I., K. P. Singh, et al. (1995). "Modulation of primary antibody response by protein A in tumor bearing mice." Immunopharmacol Immunotoxicol **17**(4): 759-73.

Protein A (PA) is a cell wall glycoprotein of Staphylococcus aureus Cowan I, which possess a number of immunomodulatory and antitumor properties. We have previously shown that PA suppresses the anti-sheep erythrocyte primary

antibody response in normal mice. The present investigation evaluates the effect of protein A on the anti-sheep erythrocyte primary antibody response in tumor-bearing mice. The primary antibody response in tumor-bearing mice immunized with sheep red blood cells (SRBC) was suppressed by the intraperitoneal administration of PA in a dose-dependent fashion. The plaque forming cell (PFC) assay was used to assess this response. Maximum suppression of the PFC response was observed at 12 micrograms PA/animal ($p < 0.001$) and could be observed at doses as low as 1 microgram PA/animal ($p < 0.01$). The amount of suppression was proportional to the number of PA doses administered. In addition this effect was critically dependent on the timing of PA administration. PA showed no significant effect on PFC when injected after immunization, but it produced pronounced suppression when injected prior to the immunization with SRBC. Maximum suppression of the PFC response was observed when PA was administered one day before the antigen challenge. PA also reduced splenic localization of 51Cr labeled SRBC to 42% ($p < 0.01$). The altered localization of antigen in spleen may be responsible for reduced PFC response in tumor-bearing mice. Depletion of B-lymphocyte is reported to exhibit tumor inhibition. Therefore, we propose that the suppression of the primary antibody response by PA helps in tumor regression by reducing the soluble immunosuppressive immune complexes.

Zeituni, A. E., W. McCaig, et al. "The native 67-kilodalton minor fimbria of Porphyromonas gingivalis is a novel glycoprotein with DC-SIGN-targeting motifs." J Bacteriol **192**(16): 4103-10.

We recently reported that the oral mucosal pathogen Porphyromonas gingivalis, through its 67-kDa Mfa1 (minor) fimbria, targets the C-type lectin receptor DC-SIGN for invasion and persistence within human monocyte-derived dendritic cells (DCs). The DCs respond by inducing an immunosuppressive and Th2-biased CD4(+) T-cell response. We have now purified the native minor fimbria by ion-exchange chromatography and sequenced the fimbria by tandem mass spectrometry (MS/MS), confirming its identity and revealing two putative N-glycosylation motifs as well as numerous putative O-glycosylation sites. We further show that the minor fimbria is glycosylated by ProQ staining and that glycosylation is partially removed by treatment with beta(1-4)-galactosidase, but not by classic N- and O-linked deglycosidases. Further monosaccharide analysis by gas chromatography-mass spectrometry (GC-MS) confirmed that the minor fimbria contains the DC-SIGN-targeting carbohydrates fucose (1.35 nmol/mg), mannose (2.68 nmol/mg), N-acetylglucosamine (2.27 nmol/mg), and N-acetylgalactosamine (0.652 nmol/mg). Analysis by transmission electron microscopy revealed that the minor fimbria forms fibers approximately 200 nm in length that could be involved in targeting or cross-linking DC-SIGN. These findings shed further light on molecular mechanisms of invasion and immunosuppression by this unique mucosal pathogen.

Zellner, G., P. Messner, et al. (1998). "Methanoculleus palmolei sp. nov., an irregularly coccoid methanogen from an anaerobic digester treating wastewater of a palm oil plant

in north-Sumatra, Indonesia." Int J Syst Bacteriol **48 Pt 4**: 1111-7.

Strain INSLUZT (= DSM 4273T) was isolated from a biogas-producing bioreactor treating wastewater of a palm oil mill on North-Sumatra (Indonesia). Cells of strain INSLUZT were highly irregularly coccoid, 1.25-2.0 microns in diameter, had a cell envelope consisting of the cytoplasmic membrane and an S-layer of hexagonally arranged glycoprotein subunits with an M(r) of 120,000, and were flagellated (motility was not observed). Cells were mesophilic and grew most rapidly at 40 degrees C on H_2/CO_2' formate, 2-propanol/CO_2 2-butanol/CO_2 and cyclopentanol/CO_2 to give methane. Tungstate promoted growth on H_2/CO_2 with acetate as the solely required organic medium supplement. The G + C content of DNA was 59 mol% (Tm method) and 59.5 mol% (HPLC method). 16S rDNA analysis revealed a phylogenetic relationship to Methanoculleus species; the name Methanoculleus palmolei sp. nov. is therefore proposed for strain INSLUZT (= DSM 4273T).

Zellner, G., E. Stackebrandt, et al. (1989). "Methanocorpusculaceae fam. nov., represented by Methanocorpusculum parvum, Methanocorpusculum sinense spec. nov. and Methanocorpusculum bavaricum spec. nov." Arch Microbiol **151**(5): 381-90.

Two new methanogenic bacteria, Methanocorpusculum sinense spec. nov. strain DSM 4274 from a pilot plant for treatment of distillery wastewater in Chengdu (Province Sichuan, China), and Methanocorpusculum bavaricum spec. nov. strain DSM 4179, from a wastewater pond of the sugar factory in Regensburg (Bavaria, FRG) are described. Methanocorpusculum strains are weakly motile and form irregularly coccoid cells, about 1 micron in diameter. The cell envelope consists of a cytoplasmic membrane and a S-layer, composed of hexagonally arranged glycoprotein subunits with molecular weights of 90,000 (Methanocorpusculum parvum), 92,000 (M. sinense), and 94,000 (M. bavaricum). The center-to-center spacings are 14.3 nm, 15.8 nm and 16.0 nm, respectively. Optimal growth of strains is obtained in the mesophilic temperature range and at a pH around 7. Methane is produced from H_2/CO_2, formate, 2-propanol/CO_2 and 2-butanol/CO_2 by M. parvum and M. bavaricum, whereas M. sinense can only utilize H_2/CO_2 and formate. Growth of M. sinense and M. bavaricum is dependent on the presence of clarified rumen fluid. The G + C content of the DNA of the three strains is ranging from 47.7-53.6 mol% as determined by different methods. A similar, but distinct polar lipid pattern indicates a close relationship between the three Methanocorpusculum species. The polyamine patterns of M. parvum, M. sinense and M. bavaricum are similar, but distinct from those of other methanogens and are characterized by a high concentration of the otherwise rare 1,3-diaminopropane. Quantitative comparison of the antigenic fingerprint of members of Methanocorpusculum revealed no antigenic relationship with any one of the reference methanogens tested. On the basis of the distant phylogenetic position of M. parvum and the data presented in this paper a new family, the Methanocorpusculaceae fam. nov., is defined.